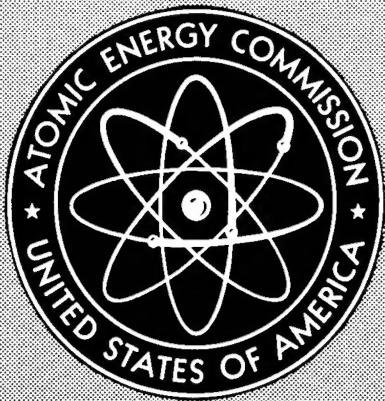


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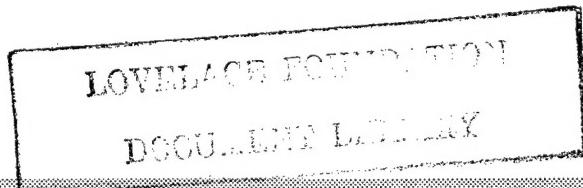
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SEMIANNUAL PROGRESS REPORT  
(ON AGRICULTURAL RESEARCH)  
JANUARY 1 – June 30, 1966

February 1967  
[DTIE Issuance Date]

Agricultural Research Laboratory, AEC  
University of Tennessee  
Oak Ridge, Tennessee

20000912021



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THE AGRICULTURAL RESEARCH LABORATORY  
operated by  
THE UNIVERSITY OF TENNESSEE AGRICULTURAL EXPERIMENT STATION  
for the  
U. S. ATOMIC ENERGY COMMISSION  
Contract No. AT-40-1-GEN-242

SEMIANNUAL PROGRESS REPORT

January 1-June 30, 1966

INTRODUCTION

Those of us who must make reports of various kinds often wonder to whom they should be directed and in what style they should be presented.

This is a progress report covering six months. One argument is that it should simply present research accomplishments and all the other business (visitors, travel, etc.) which, though it eats into our research time, is an essential part of our function. However, if this approach is taken, one wonders whether the reader gets an accurate concept of the continuity of the research or the thinking of the individuals involved.

Over the past several years our reports have become more and more detailed, relying on the fact that the limited audience was familiar with the program and would integrate the information presented into a background of previous reports. Now, realizing that with the large number of reports being produced this might drastically reduce the utility, we have modified our style of presentation.

The objective of this report, and probably those for the immediate future, will be to present the continuing program. We do this with the hope that the accumulation of data for each report period can be fitted into questions we are asking. We hope those whose needs or desires call for elaboration of a particular point will take time to call, write or visit the Laboratory. Our data and ideas, published and unpublished, are available to those with a legitimate interest.

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## Laboratory Herd Health

R. F. Hall

The overall health of the various herds and flocks maintained by the laboratory has been excellent for the current period. An average inventory of 1780 large animals--sheep, cattle, swine, and burros--was cared for.

### Miniature and European wild swine

Atrophic Rhinitis was diagnosed in the herd in 1965. Treatment of pregnant sows prior to farrowing during the fall of 1965 with sulfamethazine resulted in a group of pigs which were free clinically of signs of the disease. A few mature sows exhibiting distorted snouts still remain in the herd, but it is not known whether they may still be carriers of the disease.

Virus Pig Pneumonia has been diagnosed in the past but during this period was not diagnosed clinically.

A condition resembling exudative Epidermitis (Greasy Pig Disease) was observed in a small group of wild pigs at age 6-8 weeks. About 75 per cent of affected pigs died. Difficulty in getting wild pigs to eat commercial pig starter may be a factor in this condition. The condition is regarded as viral in etiology by some research workers. However, the disease did not spread to other groups of pigs.

### Duroc swine

A small breeding herd of Duroc swine was established in 1965 to supply animals for studies in which genetically similar animals are desired. At the time of farrowing a number of these sows experienced paralysis, difficult parturition, inadequate milk flow, uterine infections, and udder inflammation. While cultures of uterine material readily revealed infectious organisms, it is suspected that the syndrome may be triggered by border line nutritional deficiencies brought on by restricted feeding in an effort to control weight gains. The ration fed prior to and during the next farrowing period will be fortified more highly with vitamins, protein and minerals. In addition the incorporation of some antibacterial agent in the ration is expected to help correct the syndrome. Increasing maturity of the sows ought also to contribute a plus factor.

### Beef Cattle

The combination of daily observation, high level nutrition and good genetic material has produced a herd of cattle which is quite free of disease. One cow exhibited symptoms resembling grass tetany or magnesium deficiency when turned onto new pasture in April.

The tendency for Hereford cows to prolapse of vagina during late gestation or prolapse of uterus after parturition is a threat to any long term studies. Several cows have died of this condition despite persistent attempts to correct the weakness. Once the condition develops the only satisfactory solution is to send the animal to slaughter.

### Sheep flock

Nose bots and internal parasitism have been two major disease problems of the flock in the past. The flock has been dosed regularly at three-week intervals with Ruelene drench, an organic phosphate compound, in an effort to diminish the burden of these parasites. At present all sheep appear to be in good condition.

Foot rot is the third disease confronting the management of the flock. The hooves of all sheep affected have been trimmed radically and all sheep treated by standing in copper sulfate solution regularly. The number of affected animals has been reduced to four.

## PRENATAL STUDIES

### Somatic Changes in the Fetus

R. L. Murphree

That exposure of pregnant females to ionizing radiation may result in morphological defects among the offspring has been adequately documented by numerous studies in laboratory animals. Job et al. (1935) reported that the primordia of each organ system passes through a developmental stage that is particularly sensitive to radiation. Russell (1950) showed that the sensitive period for induction of limb damage in the mouse is during the early limb-bud stage. Russell and Russell (1954) and Rugh (1960) reviewed the studies in laboratory animals and Rugh (1964) postulated the sensitive periods for various defects in man by extrapolation from data in mice.

Scope:

Studies on domestic animal species in which size and length of gestation are more comparable to man than in the laboratory animal species have not been reported. We need to know (1) the effects of prenatal irradiation in agriculturally important species, (2) species similarities and differences in response, and (3) to provide a wider base for extrapolation of prenatal radiation effects in man.

Status:

The cattle, sheep, and swine in our studies were exposed in the  $^{60}\text{Co}$  facility described by Wilding *et al.* (1952) at rates of 0.3 to 0.6 R per minute. All animals received whole body exposure; radiation doses were calculated skin doses measured in air.

In cows, the midline tissue dose was estimated to be about 35 per cent of the air skin dose and for ewes and sows about 65 per cent of the air skin dose. Thus the level of radiation received by the calf embryo from a 400 R air dose was approximately twice that for sheep and swine embryos following a 400 R air dose of gamma radiation.

Following exposure of pregnant cows to single doses of 400 R between the 15th and 50th days of gestation the only morphological defects detected in the offspring at birth involved the tails and limbs. About 20 per cent of the calves irradiated at 23 to 27 days in development had crooked or shortened tails; one-half of the calves exposed on the 31st and all of those on the 32nd day of development exhibited abnormalities of the thoracic limbs. Ankylosis of the humero-radial joints was the most striking feature of the deformed legs; but associated with the abnormal joints, flexed pasterns and medial rotation of hoofs also occurred. Additionally, in one 32-day irradiated calf the femur bones failed to develop. In one calf irradiated on the 34th day of gestation the intermediate and fourth carpal bones were absent; also the pasterns were permanently flexed. No other defects were detected in X-ray photographs or at necropsy.

Only one of six calves from cows exposed to 300 R on the 32nd day of gestation exhibited limb damage. This consisted of a decrease in length of the large metacarpal bone of the left leg and fusion of the radius to the shaft of the humerus proximal to the lateral condyles; the metacarpal bone of the right leg was also shortened. Damage was not severe enough to necessitate sacrifice as was the case for the 400 R calves. No damage was detected in calves from cows which received 200 or 100 R.

Similar defects were found in lambs from ewes exposed to 200, 300, or 400 R on the 23rd-25th days of gestation. Doses of 100, 200, 300 or 400 R on the 23rd day of gestation yielded 0, 40, 60 and 100 per cent, respectively, of lambs with deformed limbs. Both front and rear legs were affected in the 24 and 25-day lambs. With the exception of the deformed limbs and shortened or crooked tails due to absence or abnormal fusion of one or more vertebrae no other damage was detected in the lambs.

Exposure of sows on each day between the 9th and 30th days of gestation to a 400 R dose resulted in shortened or crooked tails in about one-half of the pigs irradiated between 12 and 18 days of gestation. Limb damage or of any other part of the skeletal system was not seen in any of the irradiated pigs.

No evidence of abortions or of fetal deaths was seen in either cattle, sheep or swine following gamma irradiation. However, when sows were exposed to neutron radiation (dose range 337 to 406 rads) at the ORNL Health Physics Research Reactor between the 15th and 27th days of gestation, 30 per cent of the sows aborted their fetuses between 12 and 38 days postexposure. Frequency of abortions did not appear to be related either to stage of pregnancy at exposure or dose level.

Progress During Reporting Period:

Forty-one pregnant ewes were exposed at the ORNL Health Physics Research Reactor to obtain data on the response of the embryos to neutron radiation at a rate of 20 rads per minute. Of 27 ewes exposed to 200 rads between the 10th and 20th days of gestation 10 returned to estrus at varying intervals after irradiation. Among the 25 lambs produced by 16 ewes, two had shortened or crooked tails (one 16-day and one 20-day irradiated lamb). Growth of the lambs to six months of age did not differ from the growth of non-irradiated lambs.

Although evidence of abortion was not observed, the number of ewes returning to estrus (38%) after irradiation suggests an increased rate of embryonic or fetal deaths. This agrees with the findings in swine following neutron irradiation. Conversely, we have not obtained any data indicating prenatal deaths after gamma irradiation at any stage of gestation.

Of 14 ewes exposed to doses of 100, 150, 200 or 300 rads on the 23rd day of gestation, 9 subsequently produced 18 lambs. One lamb from a ewe exposed to 200 rads and four lambs (two pairs of twins) from two ewes receiving 300 rads had deformed legs similar to the damage seen in lambs exposed to gamma radiation. Additionally, both members of one pair of the twins had malshaped heads principally involving the upper jaws.

Although the numbers of animals are small, the data indicate a similar qualitative response to gamma and neutron radiation with regard to induction of limb deformities. This comparable response of lambs and the dichotomous response of pigs to the two sources of radiation leaves unanswered the questions: (1) Is this a species difference in response; or (2) is it due to differences in dose rate or linear energy transfer (LET)?

### Placental Transfer and Fetal Physiology

R. G. Cragle and M. H. Wykoff

#### Scope:

The fetal development period has great significance attached to it since it is a period of unparalleled growth and differentiation including morphologic, functional and organogenic changes. Tissue insults occurring during this period tend to be more pronounced than from similar insults at a later stage in life. A number of reports can be found on the effects of external factors influencing fetal development. Mechanisms concerning how gross treatments are expressed as fetal damage have long been alluded to but specific processes involving maternal-fetal physiological relationships are not easy to determine due to difficulties in gaining access to the maternal-placental barrier and the fetus.

The maternal-placental interface represents an almost exclusive means of transfer of nutrients to the fetus and wastes from the fetus. For this reason, the revealing of factors affecting transfer of both inorganic and organic nutrients becomes a major item for investigation.

In the case of fission products transfer to the fetus, the actual transfer from the dam to fetal circulation carries significance only in terms of the irradiation exposure to various fetal tissues. As part of these investigations it, therefore, becomes important to learn more about the irradiation dose to specific fetal tissues. This whole undertaking becomes a sizable task when one considers several species, several radioactive fission products and a number of fetal ages.

An additional goal in these investigations carries a more fundamental biological significance. During the period of fetal development, immune competence of tissues also develops. This development has a direct bearing on the future well-being of the organism and has great significance in the development of successful tissue transplantation. The classic example of natural immune tolerance

in dizygotic cattle twins clearly shows that tissue compatibility between genetically unlike animals is possible if appropriate interchange of tissue of allotypic origin takes place. Information is to be gathered from all phases of the prenatal experiments in order to conduct designed experiments in order to conduct designed experiments on this important matter at a later date.

In all of the work on prenatal physiology the dam-fetal inter-relationship must be kept in mind. The interchange of organic metabolic regulators from the dam to the fetus and vice versa must be ascertained and from this step the physiological interdependence of the dam and fetus for successful fetal development must be revealed.

Status:

A number of reports can be found in the literature on the transfer of materials across the dam-placental interface. Reports are available on blood gas exchange and carbohydrate relationships in fetal and dam bloods. The transfer of certain fission products including strontium and iodine has been reported as well as work on thyroxine exchange. Techniques such as chronic cannulation of fetal blood vessels, exteriorizing of the fetus and general fetal manipulation are known and have been used and/or improved upon to meet our particular experimental needs.

In general, for our experimental direction, the scientific literature is spares, not chronological with respect to gestation and lacks unification in areas where information is available.

Progress During Report Period:

A preliminary investigation concerned with the transfer of iodine from maternal to fetal circulation has been made in the rabbit. Transfer was studied at 6, 12, 18, and 24 days of gestation. In this study rabbits were injected with <sup>131</sup>I 24 hours prior to the time when they were anesthetized and a laparotomy was performed. At the time of laparotomy the uterus was exteriorized and the left uterine vein was cannulated while the one on the right was ligated. In conjunction to this operation the carotid artery and jugular vein were also cannulated. A tracer dose of <sup>125</sup>I was then introduced into the maternal circulation via the jugular catheter. Blood samples were taken from the uterine vein for 35 minutes after the <sup>125</sup>I dose, after which the animal was euthanized and the fetuses, fetal fluids, placenta, and maternal thyroids were collected for determination of radioactive iodine content.

From this work it appears that sizable fetal uptake of iodine takes place by the 24th day of gestation in this species. Definition between the 18th and 24th day periods is now underway.

Exploratory operations have been conducted on 5 sows and 2 ewes to determine methods and observations most applicable in these species. These procedures have included exteriorizing the fetus and performance of minor fetal surgery. In all cases the experimental aims were to return the fetuses to the uterus in a viable condition and to develop procedures for chronic investigation of fetal function. The observations of other workers and the results of our own studies to date indicate that such procedures are in the same realm of feasibility as non-uterine experimental surgical techniques. To date, adequate and efficient general anesthesia in swine has been a major obstacle.

Discussion:

This program was undertaken only eight months ago. A considerable effort has been expended during this period to become acquainted with the literature. Laboratories and work groups having information bearing on the problems of placental transfer and fetal physiology have been contacted. Experimental procedures have been revealed to us through these contacts or developed. Minor experiments have been conducted.

Our preliminary work has brought us to the point where it is felt that quantitative studies involving the transfer of radioactive tracers such as fission products and labeled organic compounds to and from the fetus via the placental interface may now be implemented.

## REPRODUCTION

### Effects of Radiation Upon Reproductive Biology

B. H. Erickson

#### Scope and Status:

Early in the history of radiation biology investigators were aware of the germ cell's particular susceptibility to the necrotizing effects of ionizing radiation. Consequently, fears arose for the loss of individual or group fertility following exposure to ionizing radiation. Of equal concern was sublethal chromosomal alterations in the germ cell that might lead to abnormal offspring. In the initial stages of more concentrated study of the effect of radiation upon the germ cell it was assumed, on the basis of limited investigations, that the process of gametogenesis was the same in all species of class mammalia and therefore the radiation response elicited by one species would be applicable to all. From studies of radiation effects conducted at this laboratory and elsewhere, however, wide species differences were demonstrated and it therefore became apparent that an extensive body of knowledge gained from studies of different species would be necessary before any reliable prediction could be made of the effect of a given dose of radiation upon a given species at some specified point in its ontogeny. Questions as to the reason for the differing responses between species led to and pointed up the need for more detailed studies of the gametogenic process in the species in question. Results of such studies have shown that the difference in radioresponse can be related to differences in the dynamics of gametogenesis. Also, as covered in the previous report (ORO-648), large quantitative differences between individuals of the large animal species have been delimited and thus call for a closer examination of the response of the individual.

#### Progress during report period:

In the principal experiment completed during this period, 48 Hereford calves aged 4 to 150 days were crated, placed on reference points in a field described by Wilding et al., and exposed to a 200-R air-dose of gamma radiation (0.7 R/min,  $^{60}\text{Co}$ ). In 3 calves, metaphosphate glass dosimeters, Cheka (1966) were fixed internally to the ovarian ligaments. Values for the dose to the ovary averaged  $61 \pm 2$  per cent of the calculated dose. Ovaries of 36 nonirradiated animals were excised either surgically or at sacrifice to serve as controls. Allocation and age distribution of animals are listed in table 1.

Upon excision, ovaries were fixed in Bouin's alcoholic solution, sectioned serially (every 20th section mounted) and stained with iron hematoxylin and orange G. Numbers and quality of primordial (oocyte with single layer of follicle cells), growing (oocyte with two or more layers of follicle cells, but without a fully-formed vesicle), and vesicular follicles were determined microscopically.

Radiation effect days 4 to 30.-- The principal histological feature of ovaries excised 30 days postirradiation was an increased incidence of atretic follicles or follicles with presumed degenerative changes within the primordial and growing categories (table 1). The approximate 5% increase in primordial follicular atresia noted at 30 days postirradiation also extended to the groups irradiated at either 40 to 60 or 120 to 150 days of age. A similar incidence of atresia was noted in both groups ovariectomized at 150 days postirradiation. Incidence of atretic changes among primordial follicles as well as difference between irradiated and control ovaries was highest in ovaries excised at approximately 725 days of age. A 98 to 99% incidence of atresia in irradiated ovaries versus 78% for the controls was apparently independent of age at irradiation (table 1).

At 150 days postirradiation, numbers of growing and vesicular follicles exceeded control values by 13 to 12%, respectively. These data are of special significance since Erickson (1966) has shown that in the bovine a positive correlation exists between numbers of primordial follicles and the other follicular classes. And since in the foregoing case the control exceeded the irradiated in numbers of primordial follicles by 17%, the likelihood of elevated numbers of growing and vesicular follicles being a radiation-induced effect is great. Similarly, the apparent lack of a superfollicular response at 30 days after irradiation may have been due to the controls' basic superiority in germ-cell numbers. In the irradiated animal, however, follicular quality apparently goes down as follicular quantity goes up, since 50 to 60% of the growing and vesicular follicles, respectively, show degenerative changes versus 30 and 44% for the controls. Increased atresia among growing and vesicular follicles was evident in nearly all treatment groups (table 1).

The superfollicular state noted at 150 days postirradiation extended to those ovaries taken at approximately 700 days after irradiation, but the probable irradiation-induced effect was found only within the growing-follicle class. Again, germ-cell numbers in nonirradiated ovaries were higher (42%) than that found in the treated ovaries.

Radiation effect days 40 to 60.--In ovaries irradiated at 40 to 60 days of age and excised 30 days postirradiation, numbers of growing follicles exceeded control values by 68% and in numbers of vesicular follicles the irradiated surpassed the controls by 52%. Here, the advantage of the irradiated population would be damped somewhat by the 13% superiority of irradiated over the control in numbers of primordial follicles.

Beyond the increased incidence of atresia among all three follicular classes, no effect was evident at 150 days postirradiation. The absence of elevated numbers of follicles in advanced stages of growth, as seen in the preceding groups and as explained earlier, was probably due to the 30% greater germ-cell endowment of the controls. At 700 days postirradiation the difference between control and irradiated groups was reflected only in a higher number of atretic follicles in the treated ovaries. Hence, when account is taken of differences in numbers of advanced follicles wrought by basic variation in germ-cell endowment alone plus values observed for ovaries in the remaining two 700-day groups, it appears that the superfollicular effect, although probably still in evidence to some degree among growing follicles, is lost sometime prior to 700 days postirradiation.

Radiation effect--days 120 to 150.--Irradiation of animals aged 120-150 days resulted in a probable 27% increase in vesicular follicles, but numbers of growing follicles were apparently unaffected. In ovaries taken 700 days after irradiation, however, numbers of growing follicles exceeded the control value by 30%. Reason for the lag in development of growing follicles could be related to the fact that ovaries aged 180 days, as compared to earlier ages (Erickson, 1966), contain a larger and presumably more responsive population of vesicular follicles; thus estrogen produced by a population of vesicular follicles expanded by irradiation would at least temporarily suppress development of growing follicles.

In a supplementary experiment, conducted in manner similar to the preceding, 15 calves aged 60 to 90 days were exposed to either a 300-R (12 calves) or 400-R (3 calves) whole-body dose of gamma-radiation. Ovaries were excised surgically from survivors at 30 to 60 days postirradiation. Two of the 12 calves exposed to 300 R and all three calves exposed to 400 R died at from 16 to 22 days after irradiation. Ovaries of heifers exposed to 400 R were characterized grossly by a mass of growing and tiny vesicular follicles ( $521 \pm 46$  and  $92 \pm 11$  irradiated,  $N = 3$  versus  $137 \pm 37$  and  $54 \pm 12$  control,  $N = 5$ ; growing and vesicular follicles, respectively). Autolytic changes subsequent to death precluded a detailed cytological analysis; but the figures listed above, as well as in table 2, reveal that the superfollicular response induced by 200 R is markedly advanced by 400 R.

Increased follicle growth generated by 300 R differed little from that seen with 200 R; however, at 30 days following exposure to 300 R, control values were only 55 and 75% of irradiated values for growing and vesicular follicles, respectively (table 2). The high rate of atresia among both growing and vesicular follicles seen at 30 days postirradiation continued to 60 days postirradiation and resulted in severely reduced populations of normal growing and vesicular follicles. In spite of a 43% advantage in primordial follicles, total vesicular follicles in irradiated ovaries were 13% below the controls and of the total of 49 vesicular follicles only 9 were apparently normal. Hence, depending upon the ability of primordial follicles to replenish growing and vesicular follicles, the consequence of this drastic reduction could be sterility.

In an experiment outlined in table 3 and instituted primarily to establish the dose of gamma-radiation necessary to kill the bovine oocyte housed in a primordial follicle, ovaries of 32 calves were exteriorized and exposed locally to varying levels of gamma-radiation emitted from a  $^{137}\text{Cs}$  source at 75 R/min.

Like the condition resulting from whole-body irradiation, the initial response of ovaries exposed to either 300 or 600 R of localized radiation was an increase in numbers of growing and vesicular follicles with 600 R being apparently more effective than 300 R. By 500 days after exposure and at both doses, however, follicle growth, in terms of follicle numbers, declined to a level slightly below that of the control. But in terms of follicle quality, nonirradiated ovaries contained 2 to 4 X more normal vesicular follicles than their irradiated counterparts.

Although 1200 R was considerably more effective, 900 and 1200 R suppressed follicle growth, when viewed both at 30 and 500 days after irradiation.

Suppression of follicular growth was all but complete and, even with primordial follicle survival, 1200 R could therefore be considered a sterilizing dose for the prepuberal bovine female. Oocytes in primordial follicles proved to be highly refractory to radiation. Effects of the 300 and 600 R levels were visible only in small increases in signs of atresia and 906 R was required to induce cell death. Following 900 R, approximately 34% of the primordial follicles survived to 30 days postirradiation versus 39% for 1200 R and 17% survived the 1200 R dose to 500 days after irradiation.

With the exception of the 3 animals exposed to 1200 R and 1 exposed to 900 R that were anestrus, all other heifers developed to 500 days postirradiation experienced first estrus within the expected age range of 9 to 15 months. Corpora lutea present in ovaries at slaughter were, however, without exception cystic or otherwise abnormal.

Discussion:

From the preceding, it is apparent that the effect of a sub-lethal dose of gamma-radiation exerts no effect on the bovine oocyte housed in a primordial follicle that can be measured immediately through assessment of cell-death. But that the primordial follicle is affected by doses within the sublethal range is evidenced by both increases in signs of atresia among primordial follicles and elevated levels of growth and atresia among the more advanced follicular classes. The significance of superfollicular growth and consequent atresia in the prepuberally irradiated female may not be great at the 200 R (whole-body) level, since the 700-day animals cycled regularly and were pregnant at slaughter; but the effects of a 300-R whole-body exposure were considerably greater than that seen with 200 R and when this is considered along with the abnormal corpora lutea resulting from localized irradiation, the question of reproductive performance among similarly treated animals looms rather large. Future experimentation is planned to answer this question, plus the correlation of germ-cell numbers with reproductive capacity. In these studies, the technic of unilateral castration (ovariectomy and orchieotomy) is being employed. Not only does this reduce the time necessary to complete an experiment, but in addition, each animal can serve as its own control.

TABLE 1. Effect of 200 R of Gamma-Radiation (Whole-Body) on the Prepuberal Bovine Ovary

Age irrad. (days)	Age cast. (days)	Primordial			Follicles (No.) <sup>a</sup> , b		
		Total	% Atr.	Growing	Normal	Vesicular	
						Atretic	
4-30	60 (8) <sup>c</sup>	100 ± 38 <sup>d</sup>	21	122 ± 15	19 ± 3	18 ± 4	
Control	60 (6)	122 ± 30	15	185 ± 49	29 ± 4	34 ± 8	
4-30	180 (5)	172 ± 48	25	121 ± 27	30 ± 9	45 ± 17	
Control	180 (12)	206 ± 52	17	154 ± 19	35 ± 6	28 ± 4	
4-30	725 (3)	73 ± 32	98	177 ± 78	21 ± 8	22 ± 8	
Control	725 (12)	125 ± 25	78	208 ± 33	25 ± 3	18 ± 2	
40-60	90 (7)	164 ± 14	26	215 ± 100	44 ± 11	60 ± 16	
Control	90 (6)	130 ± 85	28	95 ± 33	32 ± 7	22 ± 4	
40-60	180 (8)	143 ± 26	25	120 ± 34	17 ± 5	33 ± 6	
Control	180 (12)	206 ± 52	17	154 ± 19	35 ± 6	28 ± 4	
40-60	725 (8)	104 ± 27	99	115 ± 26	13 ± 2	23 ± 3	
Control	725 (12)	125 ± 25	78	208 ± 33	25 ± 3	18 ± 2	
120-150	180 (4)	272 ± 69	20	148 ± 11	62 ± 27	33 ± 5	
Control	180 (12)	206 ± 52	17	154 ± 19	66 ± 13	28 ± 4	
120-150	725 (5)	175 ± 39	98	208 ± 39	100 ± 36	34 ± 6	
Control	725 (12)	125 ± 25	78	208 ± 33	10 ± 3	18 ± 2	

<sup>a</sup>Counted in every 20th section of serially sectioned ovaries.<sup>b</sup>Primordial--oocyte with single layer of follicular cells. Growing--oocyte with two or more layers of follicle cells, but without a fully-formed vesicle.

c, d Number in parenthesis and ± denote number of animals and Standard Error, respectively.

TABLE 2. Effect of Gamma-Radiation ( $^{60}\text{Co}$ , Whole-body) on the Ovaries of Calves Aged 60 to 90 Days Postnatal

Cast. (days postir.)	Follicles (No.) <sup>a</sup> , b						Vesicular Total	
	Primordial		Growing		Normal	Atretic		
	Total	% Atr.	Total	% Atr.				
<u>300 R</u>								
30	199 ± 66 (7) <sup>c</sup>	30	59 ± 26	234 ± 74	246 ± 73	24 ± 10	68 ± 18	
Control	130 ± 85 (6)	28	95 ± 33	52 ± 4	137 ± 37	32 ± 7	22 ± 4	
60	282 ± 85 (5)	33	6 ± 4	284 ± 67	290 ± 67	9 ± 1	40 ± 6	
Control	162 ± 33 (5)	22	206 ± 53	21 ± 10	227 ± 62	29 ± 6	27 ± 2	
<u>400 R</u>								
	154 ± 28 (3)	--	---	---	521 ± 46	---	---	
							92 ± 11	

<sup>a</sup> Counted in every 20th section of serially sectioned ovaries.

<sup>b</sup> Primordial--oocyte with single layer of follicular cells. Growing--oocyte with two or more layers of follicular cells, but without a fully-formed vesicle.

<sup>c</sup> (X) and ± denote number of animals and Standard Error, respectively.

TABLE 3. Effect of Gamma-Radiation ( $^{137}\text{Cs}$ ) Localized to the Exteriorized Ovaries of Calves  
Ages 20 to 70 Days Postnatal

Dose (R)	Age cast. (days)	Primordial		Follicles (No.) <sup>a, b</sup>		
		Total	% Atr	Growing	Atretic	Vesicular
300	60-90 (5) <sup>c</sup> 520 (3)	190 ± 70 <sup>d</sup> 80 ± 14	24 90	88 ± 31 5 ± 4	244 ± 97 130 ± 56	32 ± 24 16 ± 3
600	60-90 (5) 520 (3)	197 ± 53 252 ± 94	30 90	124 ± 92 30 ± 9	480 ± 165 145 ± 48	21 ± 6 7 ± 4
900	60-90 (5) 520 (3)	43 ± 8 48 ± 7	90 95	20 ± 15 15 ± 5	127 ± 52 47 ± 8	4 ± 3 6 ± 2
1200	60-90 (5) 520 (3)	37 ± 5 19 ± 10	95 100	5 ± 2 0 ± 0	60 ± 10 10 ± 5	2 ± 1 3 ± 1
Control Days	(12)	126 ± 57	21	140 ± 41	58 ± 10	30 ± 5
Control 530 Days						28 ± 6

<sup>a</sup>Counted in every 20th section of serially sectioned ovaries.

<sup>b</sup>Primordial--oocyte with single layer of follicle cells. Growing--oocyte with two or more layers of follicular cells, but without a fully-formed vesicle.

<sup>c, d</sup>Number in parenthesis and ± denote number of animals and Standard Error, respectively.

## Lifetime Reproduction Herd

R. A. Reynolds

### Scope:

Radiation effects on reproductive performance have been studied in laboratory animals and small animals such as the dog; however, similar studies on large domesticated farm animals have not been conducted as extensively. Such studies are valuable for their basic information on gonadal and somatic tissue damage and for species comparisons. Little is known of the lifetime reproductive capacity of our larger farm animals as they are usually discarded for economic reasons. Long-term studies designed to study radiation effects on these animals can also be useful for the basic knowledge of reproductive performance in the aging animal. An experiment to study these effects was initiated in 1960 and has been called the "life-study herd".

### Status:

The life-study herd was established to observe the effects of irradiation on 1) fertility, 2) growth performance and viability of the calves, 3) pathological response in the survivors, especially latent effects, and 4) performance of the progeny by sire groups. The herd presently consists of 197 adult Hereford cows, 6 and 7 years of age, that were exposed at 18-20 months of age to whole-body irradiation from  $^{60}\text{Co}$  source. The radiation was delivered as an acute single dose for the groups receiving 200, 300, and 400 R; however, the group receiving 600 R received the dose in two exposures of 300 R each, approximately 8 weeks apart. One group was irradiated in 1960 and the other group was irradiated in 1961.

Equal numbers were started each year. The survivors were allotted to sire groups and bred at 2 years of age to calve first when they were 3 years old. Differences were noted in the mortality, from acute irradiation sickness, in the animals from different sources. At the last report period, no differences had been noted in the latent survival between the controls and the irradiated groups, nor was there a difference in the conception and calving percentage of the irradiated and the control animals.

### Contribution for the present report period:

During the report period 2 animals were lost. Both of these died with complications following parturition and as a result of complications associated with prolapse of the vagina. They were from the 300 R and 400 R irradiated group. The ovaries were recovered at the time of slaughter for histological examination.

The conception rate for all groups has been 88% up to date. The controls were slightly lower than the irradiated group with 86% conception rate. The conception rate was calculated from 839 diagnosed pregnancies of 950 matings. These resulted in 831 full-term fetuses, or a calving percentage of 87%. The number of calves raised to weaning age was 767 and represents 81% calf crop weaned for cows mated. These values compare favorably with other workers who report similar data on calves weaned. Gaines and co-workers at Virginia reported 85% conception rate with 76% of the calves raised to weaning age in a study with 3 breeds of beef cattle.

The viability and growth of the calves from irradiated dams has not been different from that of the controls.

Discussion:

The program will continue with the breeding of the survivors as long as they remain fertile and for their life span. Routine physical examinations along with hematological studies will be continued on the survivors in order to determine the occurrence of latent pathological conditions. Necropsies will be performed to observe any other pathological condition. Histological examinations of the ovaries of sterile females along with steroid analysis will be initiated to obtain additional information on the causes of infertility of these animals.

Pathology of Life Time Reproduction Herd

D. F. Johnson

Necropsy No. 3035 (300 R group):

Principal pathological changes were: partial herniation of intestinal tract (especially small intestine) into pelvic area and everted uterus; uterine and vaginal prolapse with associated inflammatory changes in the respective mucosae; and internal hemorrhage associated with rupture of some of the vascular system in the mesentery of the small intestine.

Necropsy No. 3039 (400 R group):

Salient pathological alterations were: varying degrees of apparently catarrhal endometritis, cervicitis, and vaginitis; ulcerative gastritis; catarrhal enteritis, cecitis, and colitis; and presumed septic embolism and infarcts in renal parenchyma.

## LATE EFFECTS

### Late Effects of Irradiation in Large Animals

D. G. Brown and D. F. Johnson

#### Scope:

Several groups of large animals are being maintained throughout their life-span to determine and evaluate late manifestations of irradiation effects in animals of a relatively long-life span and of sizes similar to or larger than man.

The specific objectives are to determine incidence and nature of diseases, changes in physiology of specific organ systems, longevity, cause of death, and gross and microscopic pathology as related to dose and type of radiation with time after exposure.

Data pertaining to late effects of irradiation on long-lived animals are relatively sparse compared to that of short-lived small laboratory animals; thus, data from studies such as these are necessary to bridge the gap between short-lived animals and man. In addition to the need for information on large animals from a human radiobiology standpoint, there are needs for knowledge of irradiation effects on food-producing animals which is essential not only in dealing with emergencies, but also with animal management, in the event a large percent of our livestock population is affected.

#### Method:

Each animal is subjected to physical examinations on a semi-annual basis. Such examination includes appraisal of the general physical state of each animal, hematology (hemogram, serum transaminase, serum alkaline phosphatase, blood sugar, blood urea nitrogen, and serum cholesterol), ECG (burros), urinalysis, ophthalmic examination by veterinary ophthalmologist, and fecal examination for parasitic ova and occult blood. Photographs of each animal are made at time of semiannual examinations. Weights are recorded on a quarterly basis.

The animals are vaccinated against disease, which are enzootic in this region, to which they are susceptible.

The animals are maintained on pasture and are checked each day by an animal caretaker; those which exhibit abnormal behavior are isolated for diagnosis and therapy. Therapy, when indicated, is administered only to the affected animal. Group treatment is practiced in preventive medicine and control of parasitism.

Postmortem examinations are conducted as soon as possible after death. Representative samples of tissues are fixed in 10 percent buffered formalin and prepared by the usual methods for histologic examination.

This study includes 4 groups of burros, one group each of swine and sheep. Each group will be described separately because of difference in radiation treatments as well as time of exposure. The detailed pathology is reported separately.

#### A. Whole-body Gamma Radiation (Burros)

This group consisted of 29 female burros surviving a single exposure of  $^{182}\text{Ta}$  gamma radiation in 1951 (dose range 320 to 545 R), 20 female burros surviving multiple exposures of  $^{60}\text{Co}$  gamma radiation in 1954 (total dose 375 R), 7 males surviving single exposure of 500 R  $^{95}\text{Zr}/\text{Nb}$  in 1954, and 10 female controls. The multiple exposures were administered in increments of 25 R each week at a dose intensity of 40 R/hr, and the single exposures at 25 R/hr.

#### Summary of Results to Date, June 30, 1966:

Mortality ranged from 10 percent in the 320 R group to 60 percent in the 545 R group. There have been no deaths in the controls. Survival time for the decedents ranged from 0.3 to 13.3 years with mean survival time of 7 years. Deaths have been associated with: blood dyscrasias (thrombocytopenia, 53%; leukopenia, 29%; and anemia, 18%), varying degrees of enteritis from catarrhal to fibrinonecrotic or ulcerative (47%), loss in body weight (41%), nephritis (18%), and internal and disseminated hemorrhage (12%).

The mortality of this group of burros is summarized in Table 1. No changes have occurred during this report period. One surviving animal in the 320 R group has a pronounced thrombocytopenia which has persisted for more than 2.5 years. Except for this animal, the current survivors appear to be in good health.

#### B. Whole-Body, Mixed Neutron-Gamma (Bomb) Radiation (Burros)

This group consisted of 35 burros, males and females, which survived 230 to 510 rads, whole-body exposures to prompt neutron-gamma (ratio ~ 1:1) radiation from an atomic device in 1957 and 11 male and female controls.<sup>1/</sup>

<sup>1/</sup> Response of Burros to Mixed Neutron and Gamma Irradiation  
U. S. G. Kuhn, Roy E. Kyner and Daniel G. Brown  
Radiation Res. 23: 78-93, 1964.

### Summary of Results to Date, June 30, 1966

The cumulative mortality is similar in all dose groups with an average of 45%. Nine of the 15 deaths occurred during the 15 to 25 months postirradiation. The diseases or physiological aberrations diagnosed in the 9 animals which died within the 10-month period were not different in incidence or type from the general trend: loss in body weight, 60%; thrombocytopenia, 67%; leukopenia, 33%; anemia, 20%; varying degrees of enteritis, 27%; hemorrhage in body cavity, various organs and tissues, 40%; and intestinal parasitism, 20%. One burro had nephritis and two, pneumonia. One control died with tetanus.

The mortality of this group of burros is summarized in Table 2. No changes have occurred during this report period. All currently surviving, irradiated and controls, appear to be in good health.

### C. Whole-Body Gamma Radiation (Burro foals)

Thirty-one suckling burro foals, of both sexes, 4.5 months of age, were exposed to a single dose of 250 R, whole-body, <sup>60</sup>Co-gamma radiation. These animals and an equal number of nonirradiated burro foals were divided into 3 groups and fed an iso-caloric ration containing either 9, 14, or 18 per cent crude protein. The foals were maintained on this ration during 6 to 18 months of age for the purpose of comparing the growth of irradiated and nonirradiated foals fed varying amounts of protein. After completion of the nutrition study, the animals were continued on late effects study.

### Summary of Results to Date, June 30, 1966

The foals were irradiated in 1963 and 1964, thus are currently surviving an average of 2.5 years postirradiation. The rate of mortality and cause of death have been similar in the irradiated and nonirradiated groups. The highest mortality has been in those subjected to the 9% crude protein diet. Mortality is summarized in Table 1.

### D. Whole-Body Neutron Radiation (Burros)

This group consisted of 6 male burros exposed to 180-200 rads of neutron-gamma radiation (neutron:gamma radiation, 4:1) from Godiva type reactor in 1959. There are 3 male controls. Two of 7 burros exposed to 180 rads died within 5 days after exposure.<sup>1/</sup>

<sup>1/</sup> Response of burros to neutron-gamma radiation  
Ralph E. Thomas and Daniel G. Brown  
Health Phys. 6:19-26, 1961.

No other deaths have occurred during the 7 years postexposure. The only clinical manifestation of irradiation effects apparent at the present time is graying of hair.

E. Mixed Neutron-Gamma (Bomb) Radiation (Swine)

This group of swine are survivors from an experiment designed and performed by scientists from the Walter Reed Army Institute of Research, Washington, D. C. The swine were exposed to bomb radiation at the Nevada Test Site in the Spring of 1957. The experimental design was directed toward determining the LD<sub>50/30</sub> of nuclear-weapon gamma rays, the LD<sub>50/30</sub> of nuclear-weapon gamma rays plus neutrons, and the relative biological effectiveness of nuclear-weapon neutrons.<sup>1/</sup> The survivors from this experiment were transferred from the Nevada Test Site to the Walter Reed Army Institute, Washington, D. C., where they were observed for a period of 2 years postirradiation.<sup>2/</sup> Part of the survivors were reirradiated with X-rays 4 months after the initial exposure to determine the LD<sub>50/30</sub> for a second exposure.<sup>3/</sup> When the experiment was terminated by the Walter Reed scientists, the remaining animals were transferred to this laboratory in July 1959.

The group transferred consisted of 71 surviving irradiation (30 females, 40 barrows, and 1 boar) and 35 controls (12 females and 23 barrows). The swine were of mixed breed and approximately 4 months old when exposed to the bomb. The radiation doses ranged from 15 rads to 845 rads. Only a small number in the group transferred to this laboratory were reirradiated, thus the two doses, bomb and X-ray, are added and evaluated as a single exposure. Due to the small number of animals per dose level in the high dose range, it was necessary to group these animals by average dose.

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1/ Effects of nuclear weapons on a large biological specimen (swine).

G. M. McDonnel, H. A. Claypool, W. H. Moncrief, and J. D. Goldstein  
AEC Technical Information Service Extension, Oak Ridge,  
Tennessee. ITR-1428 (November), 1957.

2/ Study of swine surviving exposure to the gamma-neutron flux of a nuclear detonation

J. N. Shively, A. R. Warner, H. P. Miller, H. J. Kurtz,  
H. L. Andrews, and K. T. Woodward  
Am. J. Vet. Res. 25:1341-1346, 1964.

3/ X-ray exposure of swine previously exposed to a nuclear detonation

J. N. Shively, H. L. Andrews, A. R. Warner, H. P. Miller,  
H. J. Kurtz, and K. T. Woodward  
Am. J. Vet. Res. 25:1128-1133, 1964.

Summary of Data to June 30, 1966

The cumulative mortality in the irradiated group is 63% and in the controls, 48%. Accumulated mortality by dose-group is summarized in Table 3. Except for neoplasia in the irradiated decedents (Table 4), the clinical and gross pathological manifestations have been similar in the irradiated and controls.

Mortality in the females appears to be related to the radiation exposures. Even though the number in each dose-group is small, this assumption seems valid. There has been virtually 100% mortality in the females exposed to doses of 250 rads and above, and approximately 70% of these have had tumors of the uteri. Approximately 25% had hepatic or intestinal neoplasia independently or along with the uterine tumors. The incidence of uterine tumors was highest during the sixth and seventh years postirradiation. Neither of the above neoplasms have been diagnosed in the controls; however, one of the controls had an apparent metastatic tumor of the ovaries.

During this report period 6 swine died, 3 irradiated and 3 controls. Each of the irradiated decedents had one of the tumors common to the irradiated swine: the 2 female decedents had tumors of the uteri and the male, tumors of the liver. No tumors were found in the 3 male controls which died.

F. Radium and Strontium Toxicity (Sheep)

This experiment was begun in 1954 for the purpose of obtaining a toxicity factor for  $^{90}\text{Sr}$  based on a comparison of radium and strontium toxicity in sheep. The experiment was designed for a short-term study; however, over 50% of the sheep dosed with radioactivity were surviving at two years and the animals were retained primarily to study the retention of the radioactive material in the body.

The lambs, of both sexes, were approximately 4 months old and averaged 27 kg in body weight at time of injection of radium and strontium. Sixteen lambs were given  $^{90}\text{Sr}$  intravenously: 4 each received doses of 0.11, 5.6, 55.5, and 278  $\mu\text{c}/\text{kg}$ . Nine lambs received intravenous doses of Ra: 3 each received 0.5, 5, and 25  $\mu\text{c}/\text{kg}$ . Six lambs served as controls.

Summary of Data to Date, June 30, 1966

Two sheep given 5.6  $\mu\text{c}/\text{kg}$  of  $^{90}\text{Sr}$  and one control are currently surviving. During this report period, 2 sheep died: one dosed with 5.6  $\mu\text{c}/\text{kg}$   $^{90}\text{Sr}$  and the other, 0.5  $\mu\text{c}/\text{kg}$  Ra. This was the last survivor of the radium group.

Three animals developed osteogenic sarcomas: one dosed with 5.0  $\mu$ c/kg of Ra at 2.8 yr, one dosed with 278  $\mu$ c/kg of  $^{90}\text{Sr}$  at 7.8 yr, and one dosed with 0.11  $\mu$ c/kg of  $^{90}\text{Sr}$  at 8.5 yr. The mortality is summarized in Table 5. The entire skeletons of those animals which have died during the past 6 years have been ashed and the total  $^{90}\text{Sr}$  content measured. An average of 5% of the intravenous dose was found in the skeleton. Measurements on the sheep dosed with Ra which died recently have not been completed.

### Discussion

Burros: The rate of mortality among the irradiated burros indicates a radiation effect. The incidence of blood dyscrasias prior to death and the similarity of these changes to early effects of irradiation in the burros lend further support to this assumption. Mortality appears to be related to dose in the gamma survivors, whereas in the mixed neutron-gamma survivors the mortality rate is similar in all dose-groups. Whether or not this is a real difference is a question which cannot be evaluated at this time due to the small number of animals involved.

The clinical and pathological findings have been similar in both the gamma and neutron-gamma irradiated survivors. The lesion of greatest significance was the decreased number of megakaryocytes in the bone marrow which usually appeared normal macroscopically. This lack of megakaryocytes corresponded in severity to the clinical thrombocytopenia. The spontaneous hemorrhages noted in a few of the cadavers seemingly were equivalent to manifestations of thrombocytopenia purpura. The significance of gastrointestinal lesions is a matter of speculation, although the similarity to lesions observed in early radiation deaths and the frequency of occurrence suggest a relationship to irradiation injury. It seems reasonable to assume that loss in body weight is secondary to other conditions; however, no correlation with the diseases diagnosed has been established.

Diseases of senility in burros have not been documented, and we have observed only a few burros older than those in this study. It is difficult, therefore, to make any judgment as to whether the changes we have associated with radiation injury are induced or temporally advanced.

Swine: The clinical and pathological manifestations in the irradiated swine differ from irradiated burros: a) no blood dyscrasias or lesions in the bone marrow suggestive of radiation damage have occurred in the swine and b) no tumors have been found in the irradiated burros. The similarities are: a) the high incidence of gastroenteritis, b) loss in body weight, c) mortality in the swine and in the burros surviving bomb radiation does not appear to be dose-related, and d) no significant changes have been found in other physiological parameters measured.

Senile changes in swine are being studied at the University of Pennsylvania and Iowa State University. These studies have been in progress for several years. A total of approximately 200 cadavers of ages ranging from 5 to 14 years have been examined at these institutions.<sup>1/</sup> The incidence of all types of tumors has been very low, less than 10%. On the basis of this information and the incidence of tumors in our experimental controls, it would seem at this time that the uterine neoplasia we have observed is related to irradiation injury.

<sup>1/</sup> Personal communication, 1966: R. Getty, Iowa State University  
H. Luginbuehl, University of Pennsylvania

TABLE 1. Late Effects of Gamma Radiation in Burros: Summary of Mortality

Dose Group	Date of exposure	No. and percent dead	Average surv. time yrs.	Median surv. time yrs.	Mean age at death years
0	9-51	0/10 ( 0%)	---		
320 R, <sup>182</sup> Ta	9-51	1/10 (10%)	7.7	7.7	10.0
425 R, <sup>182</sup> Ta	9-51	2/9 (22%)	5.8	5.8	8.5
500 R, <sup>85</sup> Zr/Nb	7-45	2/7 (28%)	1.8	1.8	6.0
545 R, <sup>182</sup> Ta	9-51	6/10 (60%)	10.0	12.1	12.8
375 R, <sup>60</sup> Co (25 R/wk)	12-53 to 3-54	6/20 (30%)	6.2	6.9	12.8
0*	1963-64	7/31 (22%)	1.4	1.7	1.8
250 R, <sup>60</sup> Co	1963-64	6/31 (19%)	1.0	1.3	1.4

\* Irradiated at 4.5 months of age.

TABLE 2. Late Effects of Mixed Neutron-Gamma Radiation (Bomb) in Burros: Summary of Mortality

Dose Group (rads)	Date of exposure	No. and percent dead	Average surv. time yrs.	Median surv. time yrs.	Mean age at death yrs.
0	9-57	1/11 (9%)	0.4	0.4	7
230 - 260	9-57	6/15 (40%)	2.2	1.9	6.5
290 - 325	9-57	6/12 (50%)	2.9	1.4	6.8
375 - 510	9-57	3/6 (50%)	2.9	1.5	6.7

TABLE 3. Summary of Mortality in Swine Exposed to Mixed Neutron-Gamma (bomb) Radiation and X-rays in 1957, July 1959 through June 30, 1966

Dose (rads)	No. and % dead			Postirrad.	Postirrad.
	Male (%)	Female (%)	Total (%)	surv. time mean (yr)	surv. time median (yr)
0	11/23 (48)	6/12 (50)	17/35 (48)	6.9	7.1
15	3/5 (60)	1/3 (33)	4/8 (50)	7.8	7.8
75	2/3 (66)	2/4 (50)	4/7 (57)	5.8	6.2
250	2/5 (40)	4/5 (80)	6/10 (60)	7.2	7.2
375	4/7 (57)	2/2 (100)	6/9 (66)	6.2	6.3
425	2/4 (50)	2/3 (66)	4/7 (57)	4.7	3.7
450*	0	3/3 (100)	3/3 (100)	4.9	4.9
480	1/2 (50)	3/3 (100)	4/5 (80)	4.8	4.5
550	2/5 (40)	0	2/5 (40)	5.7	5.7
660*	2/5 (40)	2/2 (100)	4/7 (57)	7.1	7.2
700*	2/4 (50)	6/6 (100)	8/10 (80)	6.6	6.6

\*Total dose of two exposures--the first to mixed neutron-gamma and the second to X-rays.

TABLE 4. The Incidence of Neoplasms of Uterine, Ovarian, Hepatic, and Intestinal Origin in Swine Exposed to Mixed Neutron-Gamma Radiation and X-rays in 1957 during the period of July 1959 through June 30, 1966

Dose (rads)	No. of decedents		Ovarian	Uterine		Neoplasms		Intestinal Male      Female		
				Male	Female	Hepatic				
	Male	Female				Male	Female			
0	11	6	1	0	0	0	0	0      0		
15	3	1	0	1	0	0	1	0		
75	2	2	0	2	0	0	0	2		
250	2	4	0	3	0	2	0	1		
375	4	2	0	2	1	1	0	0		
425	2	2	0	0	1	0	0	0		
450*	0	3	0	2	0	0	0	0		
480	1	3	0	2	0	0	0	0		
550	2	0	0	0	2	0	2	0		
600*	2	2	0	2	0	1	1	0		
700*	2	6	0	3	2	1	0	0		
Total	31	31	1	17	6	5	4	3		

\*Total dose of two exposures - the first to mixed neutron-gamma radiation and the second to X-rays. The ratio of neutron-gamma dose to X-ray dose was approximately 1:2.

TABLE 5. Comparison of Ra and <sup>90</sup>Sr Toxicity in Sheep:  
Summary of Mortality

Dose uc/kg	No. and % dead	Surv. time, av., yr	Surv. time, median, yr
<u><sup>90</sup>Sr</u>			
0.11	4/4 (100%)	6.3	7.4
5.6	2/4 (50%)	9.2	9.2
55.5	4/4 (100%)	4.8	5.2
278.0	4/4 (100%)	0.1	0.1
<u>Ra</u>			
0.5	3/3 (100%)	7.0	5.7
5.0	3/3 (100%)	1.5	1.5
25.0	3/3 (100%)	0.4	0.4
<u>Controls</u>	5/6 (83%)	4.8	6.6

## Pathology Report

### A. Whole-Body Gamma Radiation (Burro Foals)

Necropsy No. 2988. Significant pathological alterations included: catarrhal to hemorrhagic enteritis; renal infarct; emaciation; and verminous aneurysm and thrombosis in the anterior mesenteric and the colic arteries.

### B. Mixed Neutron-Gamma (Bomb) Radiation (Swine)

#### 1. Irradiated Swine

a. Necropsy No. 3043. Salient pathological alterations included: constipation; nonspecific catarrhal gastroenteritis, cecitis, colitis, and proctitis; catarrhal endometritis accompanied by cystic endometrial hyperplasia and apparent pedunculated and intramural leiomyomas of the uterine wall; catarrhal cervicitis; subcutaneous abscess associated with head; atrophic rhinitis; and evident bony ankyloses involving the intervertebral articulations superficially on the ventral aspects of the vertebral bodies between the fifth and sixth, sixth and seventh, ninth and tenth, and eleventh and twelfth thoracic vertebrae.

b. Necropsy No. 3050. Salient pathological changes included: slight to moderate catarrhal endometritis accompanied by moderate cystic endometrial hyperplasia; a number of intramural and pedunculated apparent leiomyomas associated with the wall of the uterus; one similar neoplasm in the wall of the cervix; bony ankyloses accompanied by varying degrees of bony exostoses over or near the intervertebral articulations from the first through the seventh and between the ninth and tenth thoracic vertebrae on their ventral aspects; atrophic rhinitis; emaciation; hydroperitoneum and hydrothorax; acute dilatation of the cardiac right ventricle.

c. Necropsy No. 3051. Significant pathological changes included: several apparent hepatomas; bony ankyloses and exostoses involving most of the intervertebral articulations on the ventral aspects of the thoracic and lumbar vertebrae; few subcutaneous abscesses; and acute dilatation of the cardiac right ventricle.

#### 2. Control Swine

a. Necropsy No. 2991. Salient abnormalities included: small, hemorrhagic focus on glans penis. (Possibly represented site of origin of prolonged, intractable hemorrhage from general area.)

b. Necropsy No. 3018. Principal pathological changes included: subpleural pyogenic infection on ventral aspects of

thoracic vertebrae from intervertebral articulation between fifth and sixth through intervertebral articulation between seventh and eighth; apparent necrosis of intervertebral discs between the fifth and sixth and between the sixth and seventh thoracic vertebrae; superficial exostoses formed on the ventral aspects of the bodies of involved thoracic vertebrae; and pulmonary edema and congestion.

c. Necropsy No. 3059. Outstanding pathological changes included: chronic subcutaneous abscess in the right flank; acute dilatation of the cardiac right ventricle.

#### C. Radium and Strontium Toxicity (Sheep)

1. Necropsy No. 3055. Principal lesions observed were: chronic ulcerative and suppurative dermatitis with possible neoplastic involvement of the skin over the ventral aspect of the sternum; evident embolic pneumonia and fibrinopurulent pleuritis.

2. Necropsy No. 3058. Significant aberrations from the normal included: pulmonary cysts indicative of infection with lungworms (Muellerius minutissimus); rumen atony and impaction; varying nonspecific inflammatory changes in the intestinal tract; chronic arthritis in both humeroradial articulations as indicated by irregularities on the articular surfaces and by apparent periarticular fibrosis; and fracture of the right humerus.

#### Late Effects of Whole-Body Gamma Irradiation on the Work Performance and Related Physiology of Shetland Ponies

D. G. Brown

##### Scope:

To evaluate the physical fitness of Shetland ponies after recovering from early effects of whole-body gamma irradiation by subjecting the ponies to work assignments and measuring various physiological changes which occur during exercise.

Studies dealing with effects of exercise on animals surviving irradiation have been limited mostly to small laboratory animals and to the period immediately after irradiation. Little is known about the physical capabilities of large, long-lived animals surviving radiation exposures. The data from this study will serve to estimate the physical capabilities of man following exposure to ionizing radiation.

Status:

Preparation for the main experimental procedures is in progress. Sixteen intact male Shetland ponies, of the same age, have been trained to work and paired on the basis of weight, temperament and behavior, and physiological factors to be measured during the experiment.

Five pairs of ponies will be subjected to work 5 days each week. The remaining 3 pairs will be maintained on pasture. The work assignment will be performed inside a building with the ponies traveling in a circular pattern. The work load, which can be adjusted by means of a valve, is generated by a hydraulic motor attached to the wheel supporting the apparatus which each team of ponies will pull. This equipment was designed and constructed by the Agricultural Engineering Department of the University of Tennessee.

One pony, 2.5 years of age, of each pair was exposed to 650 R of  $^{60}\text{Co}$  gamma radiation given in brief exposures (25 R/hr) of 50 R/wk. The animals were tentatively scheduled to have received 700 R total dose, but due to critically low platelet and leukocyte counts, irradiation was terminated (March 31, 1966) at 650 R to avoid early mortality which appeared very probable for 2 of the 8 animals irradiated.

The routine work schedule will begin 150 days after the last radiation exposure. The work load will be gradually increased over a few weeks to an average load of approximately  $5 \times 10^5$  foot pounds/hr.

The parameters to be studied will include the behavior of the animals (fatigue, change in temperament, manner of performing work, etc.), the caloric intake necessary to maintain body weight and perform work assignments, hemograms, blood chemistry (serum transaminase--SGOT), blood glucose, serum cholesterol, blood urea nitrogen, blood epinephrine and norepinephrine), and thyroid function. The heart rate, respiratory rate and body temperature will be monitored during work and during the period immediately after work while these systems are returning to the normal state. Most of the measurements will be made at 3-month intervals. Weights will be taken monthly.

Discussion:

As indicated above, the main experiment has not begun, thus no data is available other than background measurements and early response of the animals to the radiation exposures. Thrombocytopenia and leukopenia were the most pronounced changes observed. The platelet counts of 7 of the 8 animals irradiated were below 20,000/cmm (two counts were below 5000/cmm) at one week after the

final radiation exposure. Leukocyte counts averaged 2,7000 at that time. Recovery of the blood cell values has been slow. At 3 months postirradiation, the platelet and leukocyte counts averaged 50% of the preirradiation values with 2 ponies having less than 50,000/cmm of platelets.

### Tumorigenesis in Rats

J. C. Souto

#### Scope:

The scope of this work on rats, the methods and grouping have been reported previously.

#### Status:

From the data at the present time the following comments can be made:

In the female Sprague-Dawley rat, the tumor incidence is greater when injected the 7th day after birth with blood plasma from irradiated than that from normal rat or with UF from irradiated versus normal sheep.

The significance of the changes in tumor incidence in male rats injected shortly after birth on the tumor development during its life span is still questionable. Tumors were found in the pituitary, adrenals, small intestine, and colon. The incidence seems to be different but a statistical evaluation will be done at the completion of the experiment. Histological examination of the specimens obtained in the autopsies has not yet been performed.

Tumor incidence in female rats is dependent on the presence of ovaries. The tumor incidence is greater in the female rat injected shortly after birth with UF from irradiated sheep than its counterpart injected with UF from normal sheep. There does not seem to be any significant difference between the incidence of tumors of male or ovariectomized rats.

There seems to be no difference in tumor incidence whether normal UF was injected to intact or ovariectomized animals.

Males injected with plasma from either male or female donors showed little difference with the groups.

From the data we have at present, it seems that increased incidence of tumors in the female rat is confirmed when rats, under 7-days of age are injected with plasma or UF from irradiated rats

or sheep. The effect is not shown when the animals are ovariectomized. Further evaluation of the above and of the incidence in the groups of male rats will be made upon completion of the experiment which is predicted to occur during the next few months.

#### Exsanguinotransfusion in Sheep

J. C. Souto

During the period of this report, two of the sheep used in the experiments of Exsanguinotransfusions (ET) have died. Both belonged to the Irradiated to Normal group, that is, they were sheep whose blood had been replaced by that of irradiated donors. One of the sheep developed a subcutaneous tumor at both sides of the thorax and biopsy showed it was an early stage of so-called fibroepithelioma (See Progress Report, July 1-December 31, 1965). This sheep had been submitted to ET from lethally irradiated donors on July 9, 1959 and died on April 4, 1966. The autopsy showed bilateral fibrinous pleuritis and pneumonia with areas of cavitation, evident coagulation necrosis, and abscess formation in the pulmonary parenchyma (Dr. D. F. Johnson).

The second sheep that died during this period was one submitted to ET from Irradiated Donors on February 3, 1959 and died on May 6, 1966. The autopsy showed hemoglobinuria, catarrhal to hemorrhagic enteritis, cecitis, colitis, and proctitis, and terminal respiratory failure (Dr. D. F. Johnson).

## EARLY EFFECTS

### Dizygotic Twin Cattle and Tissue Tolerance

R. G. Cragle

#### Scope:

Reports on this project have appeared in previous progress reports (ORO-605, 614, 624, 625, 631, 635, and 648). Thirty-five sets of twins were purchased in 1962 and 1963. These twins are unique in that they are fraternal and are natural chimeras. These natural chimeras exhibit a number of characteristics which make them extremely useful animals for study of basic biological phenomena. First, it has been established that they are chimeric for red cells, and white cells and perhaps a number of other tissues. Secondly these animals exhibit tolerance to each others tissues which is a basic biological phenomenon of great significance in the field of tissue and organ transplantation.

Our current intent is to reveal as much detail as possible concerning the chimeric condition.

#### Status:

This project has now been active for four years. A number of important facts have been established. It is now known that the red cell equilibrium in these chimeras can be shifted by whole-body gamma irradiation<sup>1</sup>. Initially single large doses were used but it has now been established that shifts can be made with complete predictability (but not the direction of occurrence) by using a fractionated whole-body gamma irradiation treatment (Figure 1). This can be accomplished without inducing the irradiation blood syndrome of reduced white cells and platelets and therefore without inducing the usual clinical symptoms of irradiation treatment. It also has been established that skin grafts between co-twins do not reject in the same manner as grafts between other genetically unlike cattle. Although skin grafts between animals of the latter type reject in less than 12 days, grafts between chimeric twins remain for many days and when the rejection does occur it is far less violent than in the nonchimera case. Even more important, rejection has not occurred in some cases three years after the grafts were made. The success of the skin grafts stimulated efforts to make organ grafts. This program was initiated in September 1965.

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<sup>1</sup>/ These animals have one red cell type corresponding to their own genotype and a second red cell type corresponding to their co-twin genotype.

Two basic areas of work are before us in this project. The first involves revealing as many facets as possible concerning the chimeric condition and its related phenomenon of tissue tolerance. The second area involves the mechanism of producing tolerance. The initial experiments aimed at this latter goal will be initiated in early 1967.

Progress During Report Period:

A. Kidney Transplants: Reciprocal kidney transplants to the neck were made between four sets of chimeric twins during the last report period. An additional two sets of twins were grafted during this period. In addition, two sets of non-chimeric genetically unlike animals were grafted. These data are summarized in Table 1. Eight of the chimeric kidney transplanted animals have functional kidneys which have performed satisfactorily for from 10 to 36 weeks. One autoplant has functioned for 54 weeks. The four non-chimeric genetically unlike animals rejected their kidney grafts within twelve days as was expected.

B. Reasons for Red Cell Shifts: With various irradiation treatments the red cell proportions (a shift of percentage of its own cells vs. co-twin cells) in chimeric twin cattle's blood shift (Figure 2). This may occur for one or more of three reasons. First, the stem cell population may change with treatment. Secondly, one cell type may be proliferated (from stem cells to peripheral cells) in a different manner. Thirdly, the peripheral life span of one cell type may be different. Work with radio selenium and iron has been initiated to gain information on the latter two possibilities.

C. Treatment with 25 R/hour for long periods of time: Irradiation of the ten animals in group 10 at the rate of 25 R whole-body gamma per week for 40 weeks (100 R) was completed during the last report period. Without exception, major shifts in the red cell proportions have occurred in all of these animals.

D. Infusion with antibody: The possibility exists that circulating antibody may account for a change in red cell proportions has been checked for a second time. Antibody was infused into the experimental chimeric animals at a slow continuous rate over twenty hours (two and one-half 8-hour days). As we found before, the cell type which was attacked by the antibody showed a marked decrease in abundance in relation to the remaining cell type.

E. Karyotypes: Additional karyotypes of white cells on group 10 animals (unlike sex twins) have been done, both here and at Cornell University. The most important finding is that red cell proportion shifts are not reflected in white cell shifts. These data are given in Figure 4.

Discussion:

Much remains to be done to reveal the various aspects of the chimeric state. Major efforts during the near future will be centered on determining how well transplanted kidneys function. Work is continuing to reveal more about red cell kinetics and why the cell proportions shift. One of the most important items to be initiated is development of an in vitro leucocyte test for tissue compatibility between donor-host.

Acknowledgement:

This study was initiated by and is conducted in cooperation with W. H. Stone, Department of Genetics, University of Wisconsin.

TABLE 1. Preliminary Results from 17 Kidney Grafts in Cattle

<u>Reci- pient</u>	<u>Donor</u>	<u>Type of Graft</u>	<u>Date</u>	<u>Status on June 30, 1966</u>
374	374	Autograft	6-15-65	Functional at 380 days.
111	112	Co-twin	9-13-65	Functional at 295 days.
112	111	Co-twin	9-13-65	Functional at 295 days.
135	136	Co-twin	9-15-65	Died immediately after surgery, 9-15-65.
136	135	Co-twin	9-15-65	Functional for 66 days, infec- tion, anuria, died 11-20-65.
145	146	Co-twin	12-28-65	Non-functional - anuria; removed 4-15-66.
146	145	Co-twin	12-28-65	Functional at 183 days.
147	148	Co-twin	12-30-65	Functional for 61 days, infec- tion, anuria, removed 4-21-66.
148	147	Co-twin	12-30-65	Functional at 181 days.
139	140	Co-twin	4-13-66	Functional at 78 days.
140	139	Co-twin	4-13-66	Functional at 78 days.
149	150	Co-twin	4-15-66	Functional at 76 days.
150	149	Co-twin	4-15-66	Functional at 76 days.
184	705	Allograft	6-13-66	Rejected within 12 days.
705	184	Allograft	6-13-66	Rejected within 12 days.
712	713	Allograft	6-15-66	Rejected within 12 days.
713	712	Allograft	6-15-66	Rejected within 12 days.

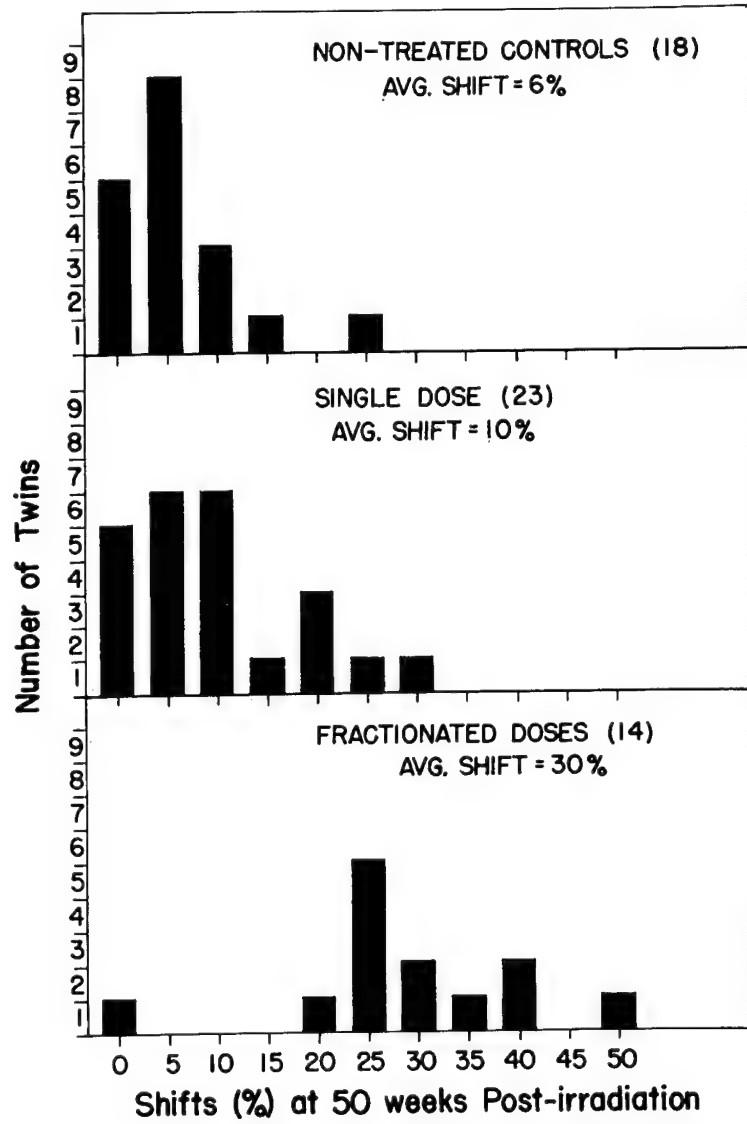


FIGURE 1. Effects of Gamma-Irradiation on Proportions of the Two Cell Types in Chimeric Cattle Twins

FIGURE 2

**SHIFTS\* IN THE PROPORTIONS OF BLOOD TYPES  
IN IRRADIATED CO-TWINS**

Number of Twin Pairs Showing:	
Same Behavior	Different Behavior
No shift in both twins	Same shift in both twins
3	10

Shift in one twin only      Shift in opposite directions

3      0

\* Shifts refer to direction  
All shifts greater than 10%

FIGURE 3

DISTRIBUTION ACCORDING TO SHIFT (%) IN  
CELLS REPRESENTING TWIN'S GENOTYPE

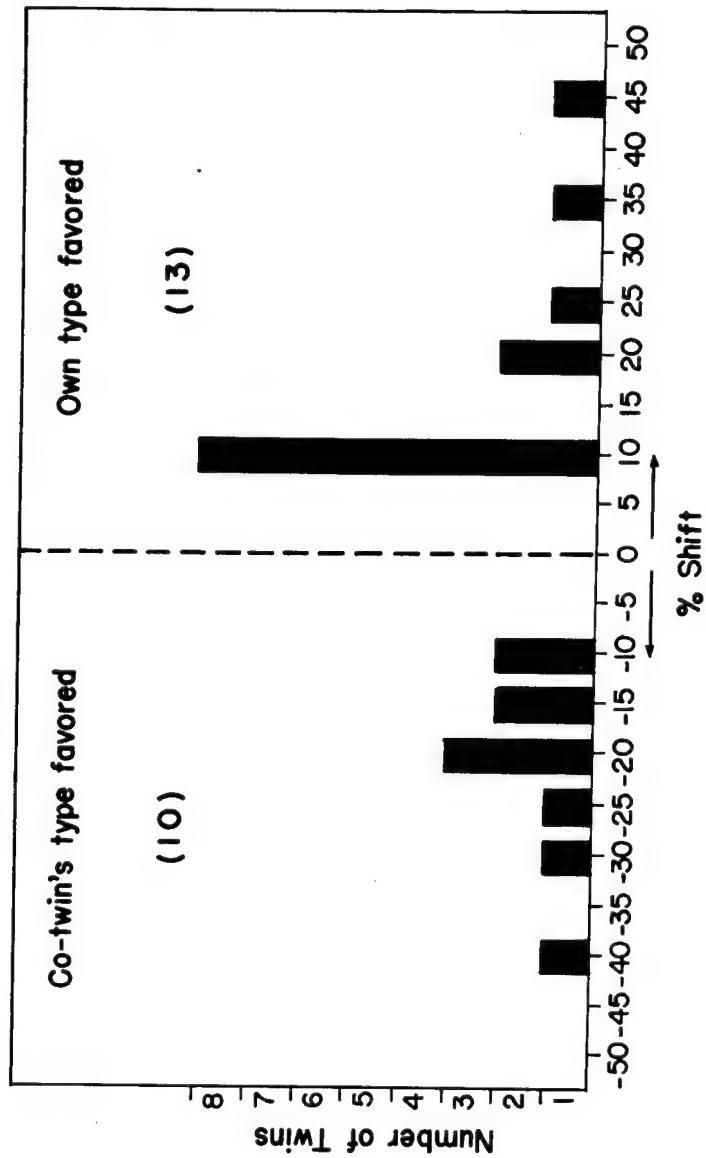


FIGURE 4

COMPARISON OF KARYOTYPE AND ERYTHROCYTE  
CHIMERISM IN CATTLE TWINS

TWINS NO.	SEX	KARYOTYPE *		ERYTHROCYTE **	
		XY	XX	I	II
161	♀	72	28	73	27
162	♂	42	58	66	34
163	♀	51	49	60	40
164	♂	57	43	63	37
165	♀	21	79	32	68
166	♂	12	88	25	75
167	♀	51	49	80	20
168	♂	40	60	70	30
169	♀	40	60	95	5
170	♂	21	79	95	5

\* AVERAGES (%) BASED ON COUNTS OF 6 SAMPLES TAKEN AT APPROXIMATELY  
MONTHLY INTERVALS. AT LEAST 50 CELLS COUNTED FOR EACH SAMPLE.

\*\* AVERAGES (%) BASED ON 10 DIFFERENTIAL HEMOLYSIS TESTS MADE DURING  
THE SAME TIME PERIOD.

## End Products in Blood and Urine Following Whole-Body Irradiation

J. C. Souto and P. G. Martin

### Scope:

Various experiments with high-energy radiation on dilute organic and aqueous solutions has proven the formation of free radicals. The radicals formed are different under aerobic and anaerobic conditions. In working with what he called a manometric actinometer, Warburg showed that in irradiation of living cells two oxidants resulted from the splitting of water. The author states "the two oxidants, the hydrogen peroxide and OH-radicals may oxidize every component of the living cell". Other experiments by Dale have shown the destruction of activity of dilute enzymes through diffusion of radicals; the assumption is that the same process works in the living cells. On the basis of these and other findings, the formation of unique molecules from radicals formed by radiation is entirely possible. These end products, if formed, should be found in the blood and urine. Our work has been directed toward showing that some unique substances are present in the blood and urine of animals after receiving whole-body irradiation (WBI).

### A. Urinary Components:

#### Status:

During previous work, six different substances have been found in the urine of irradiated sheep, burro, rabbit, rat, man and pig. Among these, one in particular (see Progress Report, July 1-December 31, 1965) has been repeatedly demonstrated in the urine of irradiated animals and man, with the exception of three leukemic patients.

#### Progress during report period:

In this six-month period, verification of this specific end product has been established in three additional sheep receiving WBI. Verification in urine collected up to twenty-two days post-irradiation was carried out using Thin-Layer Chromatography (TLC). Standardization of our procedure during this period which will allow for the isolation and identification of these substances has taken considerable time to develop. The methods used for extraction and isolation, although systemized, require a long time.

Standardization of our procedures during this period have made it easier to obtain reproducible results and will expedite future analysis.

At the present we are concentrating our effort on the verification of our former work using more rapid extraction procedures.

#### B. Blood Cell Proliferation Studies

These experiments are closely related with the finding of substances in urine formed in animals after WBI. In preliminary work, several end products of a non-protein nature were found in pooled samples of normal sheep blood and in blood collected between the first and seventh day post-irradiation.

Former ET experiments showed changes in the myeloid cells of both bone marrow and peripheral blood occurred when normal sheep blood was replaced by another from a lethally irradiated donor. We tried to check whether some humoral factor might be responsible for those changes. Besides the reduced amounts of blood fraction obtained from irradiated and normal sheep when injecting that fraction into animals, the introduction into a living animal of foreign proteins produces stress, variability of blood counts, etc. Because of these factors in vitro procedures were undertaken.

Contrary to the results of Gidali, experiments in which  $^{14}\text{C}$  was incorporated into bone marrow cells of rats and rabbits did show a lineal relationship after 24 hours of incubation of bone marrow cells with  $^{14}\text{C}$  sodium formate. In new experiments, great differences were found in the  $^{14}\text{C}$  sodium formate. In new experiments, great differences were found in the  $^{14}\text{C}$  counts per a given number of cells. This test, therefore, could not be used for checking material acting upon blood cells. Mitosis counts in blood incubated in vitro with blood plasma or its fractions from irradiated and normal animals also gave erratic, inconsistent results.

A procedure based upon the synthesis of DNA in vitro as a measure of cellular proliferation (Bond, Cronkite, et al.) was developed. Tritiated thymidine (3-Thy.) was added to suspensions of rabbit bone marrow and incubated for periods between 40 and 60 minutes. High resolution autoradiographs were made. The number of cells of each series which had incorporated 3-Thy. were counted and both total and differential counts on the number of tagged cells was performed. One thousand cells were counted for every fraction tested and for each total or differential counts. Positive cells were considered the ones in which at least four granules were shown in their nucleus. Background was calculated as the number of granules (from a number of four and up) in similar-sized areas as estimated by a reticulum.

The results of this experiment showed the blood ultrafiltrate (UF) obtained from normal sheep showed some inhibitory effect on the number of cells incorporating tritiated thymidine as compared with both UF from irradiated sheep or with tubes

having no UF. The decreased number in tagged cells is shared by both the elements of the myeloid series and those of the erythroid series.

In order to know whether the effect on cell proliferation as shown by UF from normal but not from irradiated sheep might be related with some of the substances detected in UF from irradiated animals, each UF was submitted to further fractionation by preparative TLC. Of course, it should be stated here that every fraction, whether extracted by water or not, was always in an aqueous solution when tested for cell proliferation in vitro.

Results of these experiments showed that possibly one of the fractions obtained from normal UF (the so-called 2-Ac.ac.) may have an inhibitory effect on the  $^{3}\text{Thy}$ . incorporation into bone marrow cells. Some other extracts of the fractions 1 and 2 showed a distinctive increase of cell proliferation as compared with both irradiated and non-irradiated UF. This increase of cell proliferation by UF from normal sheep is shared by cells of both series, myeloid and erythroid.

These results should be interpreted cautiously, not only because of the small number of experiments performed but also because in the case of increased cellular proliferation in normal UF there was a great destruction of cells. It is true that the destruction occurred equally in the tubes with normal or with irradiated UF which seems to indicate the reason for that is other than the above mentioned effect just in the normal UF.

In the future, experiments are to be performed in trying to verify the effects of fractions from UF from normal and irradiated sheep on proliferation of bone marrow cells. We are trying to show whether differences in some components can be shown between UF as obtained from irradiated and normal animals, not only by chemical analysis, but also by the possible effect of some of the blood components on the proliferation of bone marrow cells.

## TISSUE CULTURE

### Tissue Culture

A. F. McFee

#### Scope:

Encompassed in the tissue culture area are efforts to establish the patterns of normal behavior for domestic animal cells under in vitro conditions and to investigate the changes in these patterns produced by exposure of the animal or its cells to ionizing irradiation. Techniques are being developed for the in vitro maintenance and growth of cells from various tissues of domestic animal species and to establish the normal chromosomal make-up of these cells. Through these methods preparations can be obtained which permit the direct examination of the chromosome complement of a cell for the evaluation of radiation-induced or naturally occurring changes. With the addition of radioisotope labeling methods, changes in the time and rate of DNA synthesis can be detected as well as the grossly visible chromosomal aberrations. Radiation-induced chromosomal aberration rates in the leucocyte offer the possibility of a built-in biological dosimetry system which is readily accessible and which should have received an exposure dose representative of the average of the animal's tissues. The variations in individual animal sensitivity to radiation may also be amenable to tissue culture methods; if the susceptibility of an animal's cells to in vitro irradiation can be correlated to the sensitivity of the animal itself then prediction of resistant animals would be feasible. This prediction ability without the exposure of the animal would permit the development of radioresistant and sensitive lines of animals. Through the study of cells in culture we hope to gain some insight into the effects of radiation on metabolic processes and the mechanisms of damage in particular organ systems. Of particular interest is the demonstrated fact that certain systems within the developing embryo are unusually susceptible to damage at particular times during gestation. An integral part of the work in this area will be comparisons between species wherever such a course seems warranted.

#### Status:

Methodology for the culture of peripheral blood leucocytes was first established in the field of human medicine and has been applied to domestic animal studies to only a limited extent. Although additional study is needed relative to the growth requirements of leucocytes from various species, successful cultures can be obtained from sheep, cattle, horses and especially swine. The ease of culture and relatively small number of chromosomes in pigs make them the preferred animal for many trials. Previous

experience has indicated that the expected chromosome aberrations appeared in pig blood following in vitro irradiation but that following in vivo irradiation these aberrations did not persist as they have been shown to do in man. This seemed to necessitate some investigation of the basic differences between cells of the two species.

The unusual chromosome complement in European wild pigs has been previously reported from this laboratory. Fertile offspring are obtained from matings between wild pigs with either 36 or 37 chromosomes and domestic breeds which routinely have 38. Chromosome number in the progeny is, of course, determined by the parental mating and has thus far followed a predictable Mendelian-based pattern.

Progress During Report Period:

Some 1012 leucocyte cultures were performed during the current report period. The majority were relevant to one of three general areas: improvements in culture techniques, the inheritance of chromosome number in wild pigs, and the kinetics of swine cells in culture. Blood serum from adult burros continues to show promise as an ingredient of culture media in place of the more expensive fetal calf serum. Bovine samples respond especially well, yielding consistently higher mitotic indexes in burro serum, while leucocytes from sheep, swine and human subjects grow equally well with either serum. While it is impossible to quantitate the "quality" of metaphase spreads in these cultures, we feel that the advantage is with samples grown in burro serum media. Improved spreading of metaphase configurations in all samples has been brought about by an additional step in their preparation. Following fixation and one or two washings of the cells in 3:1 alcohol:acetic acid we find that additional chromosome dispersion without cell rupture can be obtained by a final suspension in a 1:1 mixture of the two ingredients.

During mid-May of this year we experienced a sudden deterioration in the quality of cultures from all species which we are at a loss to explain. Systematic changing to new lots of all materials used in the procedure as well as numerous variations in technique failed to yield any significant improvements. After some 5-6 weeks of extremely poor samples, the quality of our results gradually returned to normal. Seasonal variation of some blood constituent in the animals is suspected although no direct evidence is available.

Studies to determine chromosome number and its mode of inheritance in European wild pigs now contain data from approximately 175 animals. Sufficient numbers have been obtained to show conclusively that: 1) crosses between wild pigs both of which have 36 chromosomes produce only 36-chromosome offspring; 2) 36 x 37-

chromosome matings yield pigs with either 36 or 37 in approximately a 1:1 ratio; 3) all progeny from 36 x 38-chromosome crosses possess 37. Additional breedings have been made of 37 x 37 and 37 x 38 crosses. As an aspect of this study, samples were secured (courtesy Dr. N. M. Kieffer, Texas A and M University) from three Piney Woods wild pigs in Louisiana. These animals had the same 38-chromosome complement characteristics of domestic pigs.

Blood cultures from several irradiated pigs failed to exhibit chromosome aberrations persisting for long periods after exposure such as those found in humans. Investigations are in progress which are aimed at finding the basic differences between cells of the two species which would account for the dissimilarity of aberration persistence between them. Initial efforts have been toward defining and gathering basic information on the kinetics of swine leucocytes in culture. These parameters will then be compared to those already available for human cells so that similarities and differences may be defined and more meaningful extrapolations between the species may be made. Through the use of cell labeling techniques employing tritiated thymidine, some preliminary values have been obtained relative to the division cycle of the pig leucocyte: mitotic cells begin to appear in appreciable numbers about 30 hours after initiation of the culture and reach a maximum number at about 60 hours. DNA synthesis does not occur prior to 12 hours in culture, is noticeable at 18 hours and reaches a peak at about 60 hours. Present estimates are that the G<sub>2</sub> phase of the initial *in vitro* division cycle lasts from 3 to 5 hours and that S plus G<sub>2</sub> spans approximately 23 hours but may be as long as 36 hours in some cells or under certain conditions. Studies in progress will more precisely define the behavior of swine cells in culture prior to making comparisons between the irradiation response of human and pig cells.

#### Discussion:

Tissue culture techniques should be applicable in many studies relative to radiation effects, metabolism and genetics in domestic animals. The development of reliable procedures and the establishment of normal parameters are, of course, prerequisite to their adoption. The efforts in this laboratory will continue to be directed toward new and improved methodology and especially toward the application of these methods in gathering basic data on the kinetics of domestic animal cells in culture. This information will then serve as a basis for evaluating radiation-induced changes in cell morphology and behavior. Special interest situations such as the wild pigs will continue to be explored whenever they show promise of yielding useful information.

## PATHOLOGY

D. F. Johnson

During this period, 60 cadavers from five families have been subjected to macroscopic examinations by pathologists. The number examined included 13 cattle, 3 burros, 15 sheep, 27 swine, and 2 rabbits. Outstanding pathological manifestations relative to the necropsies of irradiated animals are incorporated into the reports from the respective studies where appropriate. The pathology of animals from miscellaneous programs is:

### A. Cattle

#### 1. Necropsy No. 3003 (newborn calf - prenatal studies)

Pathological manifestations were: subcutaneous hemorrhage and edema in ventral cervical and ventral cephalic areas; a moderate amount of serosanguineous fluid in the pleural cavity and the pericardial sac.

#### 2. Necropsy No. 3038 (prenatal studies)

Significant aberrations from the normal included: moderate pulmonary edema and congestion; a moderate amount of serosanguineous fluid in the pericardial sac; markedly hyperemic to hemorrhagic vaginal, cervical, and uterine mucosae associated with uterine prolapse.

#### 3. Necropsy No. 3045 (prenatal studies)

Principal pathological manifestations were: considerable subcutaneous and intermuscular hemorrhage and edema on the left side; a marked accumulation of serosanguineous fluid in the pericardial sac; and considerable pulmonary edema and congestion.

#### 4. Necropsy No. 3036 (Stone project)

Outstanding pathological changes were: marked epilation accompanied by apparent dermal and subcutaneous fibrosis on the irradiated (left) side; aspiration of regurgitated ingesta into lungs; evident rumen atony; suppurative endometritis; and subacute suppurative mastitis.

#### 5. Necropsy No. 2992 (Brookhaven cattle)

The outstanding pathological changes were: vegetative valvular endocarditis and ankylosis of the right elbow joint.

6. Necropsy No. 3054 (Brookhaven cattle)

Principal pathological changes included: suppurative cystitis, ureteritis, and nephritis; and tendinous and muscular rupture over the right tuber coxae and the posterior aspect of the right thigh.

B. Sheep

1. Necropsy No. 2990 (general herd)

The outstanding pathological features were hydrothorax and pneumonia.

2. Necropsy No. 3004 (general herd)

The principal abnormalities observed were: dilatation of some components of the urogenital tract with mucoid material; dilatation of the lower bowel with mucoid material and meconium; and agenesis of the anus and vulva.

3. Necropsy No. 3009 (general herd)

The most noteworthy pathological manifestations included: the unhealed stump of an amputated tail (possible source of tetanus); necrotizing pneumonia; and fibrinous pleuritis.

4. Necropsy No. 3011 (nutrition studies)

Pathological alterations included: apparently hemorrhagic and necrotic medulla in the right adrenal; focal necrosis in the hepatic parenchyma; and pulmonary edema and congestion.

5. Necropsy No. 3016 (nutrition studies)

No significant lesions were observed on cursory examination.

C. Swine (nutrition studies)

The pathologist supervised evisceration and observation of only four cadavers (Necropsy Nos. 3005, 3006, 3007, and 3008). Only Necropsy No. 3008 displayed any significant gross deviation from the normal. This condition was a bilateral bronchopneumonia.

Other Activities:

The Histology Laboratory has prepared a total of 10,080 slides of tissues for microscopic evaluation. Involved in the preparation of at least a portion of the slides for histopathological examination were a number of special stains: Brown and Brenn's

method for bacteria; Lillie's quick modification of Gram's stain for bacteria; Weil's method for myelin sheath; Gomori's reticulum stain; Gomori's chromaffin stain; May-Grunwald-Giemsa's stain for mast cells; periodic acid-Schiff reaction; Gomori's iron reaction; Ziehl-Neelsen stain for acid-fast bacteria; Warthin-Starry method for spirochetes; and Gomori's methenamine-silver nitrate technic for fungi.

In addition, the Histology Laboratory prepared about 620 micro-autoradiograms.

## NUTRITION

### Swine, Sheep and Beef Cattle Phase

M. C. Bell

#### Scope:

The purposes of this program are to study (1) the metabolism of fission products and chemically related nuclides among species and (2) the effects of irradiation on the metabolism, in animals, of mineral elements.

Comparisons among species to determine metabolic differences and similarities are used to give direction for subsequent detailed investigations. The information gained will be of benefit in providing a better understanding of interactions on both food nutrients and undesirable food constituents among several species. Primary emphasis is on animal species supplying the major sources of dietary protein in the United States. Irradiation effects data are needed from both internal and external sources in species more closely related in size to man in order to evaluate data among several animal species. These include chronic and acute exposures to both absorbed and nonabsorbed fission products. Radioisotope tracer data are also needed on mineral elements to explain the wide variation in requirements and chemical toxicities as affected by species, dietary changes, and route of administration. Detailed biochemical data are needed to explain these differences.

#### Status:

Both whole-body irradiation from gamma rays and gastrointestinal irradiation from high levels of a poorly absorbed (0.05%) fission product,  $^{144}\text{C}$ - $^{144}\text{Pr}$ , increased absorption and retention of  $^{89}\text{Sr}$  with no observed effects on  $^{45}\text{Ca}$ .  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  accumulates in liver with eventual translocation to bone from both oral and intravenously administered radioactivity. Oral administration of

high levels of  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  had no significant effect on digestibility of either roughage or concentrate rations in sheep. Both acute and chronic radiotoxicity of  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  have been studied showing the LD<sub>50</sub>/100 for a single intravenous dose to be around 50  $\mu\text{c}/\text{kg}$  of body weight in sheep. Deaths are due to low platelet and white cells with no effects on erythrocytes until blood loss from excess hemorrhages.

Whole-body gamma irradiation of pigs at 450 R temporarily increased  $^{89}\text{Sr}$  absorption and retention but had no significant effect on  $^{45}\text{Ca}$  metabolism. When these pigs were dosed 21 days after irradiation, no differences were found comparable to those observed one day after irradiation. Whole-body gamma irradiation of sheep and swine at 400 to 450 R at the rate of 0.4 R/min had no significant effect on the absorption and metabolism of  $^{99}\text{Mo}$ . Most  $^{99}\text{Mo}$  was excreted in 24 hr in urine of intravenously and orally dosed pigs while cattle excreted most of the  $^{99}\text{Mo}$  in feces. Sheep reacted similarly to cattle except that the amount of roughage in the ration affected  $^{99}\text{Mo}$  metabolism. Efforts to explain the species differences in  $^{99}\text{Mo}$  metabolism using  $^{35}\text{S}$ -sulfate uptake and total mucopolysaccharides in the intestinal mucosa and kidney have involved the establishment of acceptable techniques based on similar analyses in blood platelets.

In vitro uptake of radioisotope tracers by erythrocytes as a measure of trace mineral deficiencies has been expanded to include animals exposed unilaterally to neutron irradiation. Preliminary data show that hair color is associated with the content of zinc and copper.

Life span of erythrocytes, using  $^{75}\text{Se}$  as a marker, was found to be about 150 days in sheep. Data on a limited number of animals showed that this procedure did not work for swine.

Establishment of carbohydrase activity in the intestinal tract of mature sheep shows that the levels are much lower than in simple stomach animals. The jejunum contained the greatest amount of enzyme with maltase being the most abundant enzyme.

Progress During Reporting Period:

In trying to determine the persistence of the effects of whole-body irradiation on  $^{89}\text{Sr}$ , it was found that swine dosed 7 days after whole-body irradiation metabolized  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  the same as the controls. Those dosed one and 3 days after irradiation showed a reduced uptake of both  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  in plasma for the first 4 hr, but after 8 hr, plasma levels persisted higher than both the controls and those dosed 7 days after irradiation as shown in Figure 1. Tissue repair and regeneration of the

intestinal mucosa in less than 7 days offer logical explanation for the recovery of normal absorption of radiostrontium.

Chronic radiotoxicity from intravenous  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  administered in July and August 1965 has resulted in the death of 3 more sheep during this reporting period. Blood data show that they died after a prolonged drop in white cell and platelet fractions of the blood. After over 330 days, there are 19 survivors of a group of 30 sheep given a single intravenous dose at levels ranging from 0.125 to 2 mCi of  $^{144}\text{Ce}$ - $^{144}\text{Pr}$ . Sheep fed high levels of  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  (20 mCi daily) for up to 60 consecutive days do not show these changes.

Irradiation to the surface of the gastrointestinal mucosa has been estimated to be up to 1000 R daily from these weak gamma and beta rays. Thus these animals, as has been shown before, illustrate that the bone marrow cell is more sensitive to irradiation than the gastrointestinal mucosa.

In trying to explain the blood data from one sheep which metabolized  $^{99}\text{Mo}$  quite differently from the others in a group of 15, it was found that  $^{99}\text{Mo}$  given directly into the abomasum was absorbed much more quickly and metabolized differently than orally given  $^{99}\text{Mo}$ . Data from these sheep confirm the data from the one sheep which indicated the gelatin capsule of radioactivity had quickly left the reticulum and gone into the abomasum. Sheep and cattle fed identical rations metabolize  $^{99}\text{Mo}$  very similarly, but sheep and swine fed identical rations metabolize  $^{99}\text{Mo}$  quite differently.

$^{99}\text{Mo}$  given as ammonium molybdate directly into the abomasum of sheep is quickly absorbed, as is the case in swine. Since the abomasum is quite similar to the pig stomach with secretion of HCl, we expected similar metabolism. However, blood levels were similar for only 4 hours. After 4 hours, the blood was rapidly cleared of radioactivity by urinary excretion from the pig. These data show a definite difference in renal clearance of  $^{99}\text{Mo}$  by swine and sheep. The differences in  $^{99}\text{Mo}$  metabolism from oral dosing and abomasal dosing of sheep may be due to alteration of the ammonium molybdate into a form which is poorly absorbed. But once  $^{99}\text{Mo}$  goes into the blood stream of sheep, it persists at measurable levels for at least 7 days compared with only 2 days in swine. Bacterial action in the rumen is probably responsible for changing molybdate either into usable enzymes such as xanthine oxidase or complexed into non-absorbed organic and inorganic compounds.

It has been shown, then, that  $^{99}\text{Mo}$  metabolism is affected by species, route of administration and ration ingredients.

Since dietary sulfate has been shown to affect molybdenum metabolism, biochemical and radiochemical techniques are being tested and improved to study the sulfated and total mucopolysaccharides in kidney and intestinal mucosa among the 3 species in trying another theory to explain the differences in  $^{99}\text{Mo}$  absorption and metabolism.

Research is continuing on in vitro uptake of radioactive trace minerals by erythrocytes as affected by ration and neutron irradiation of swine. The possibility of using this technique as a clinical test for mineral imbalances and deficiencies is obvious. Analysis of stable zinc and copper in hair and skin, and metabolism of oral  $^{65}\text{Zn}$ , are also being studied since zinc is associated with tissue repair and color is usually associated with copper in hair. Neutron irradiation retarded hair growth and dark hair was replaced by white hair.

In determining the differences of  $^{75}\text{Se}$  retention in erythrocytes between swine and sheep, a study has been initiated to compare the binding of  $^{75}\text{Se}$  to several fractions of erythrocytes and plasma. This includes chromatographic and electrophoretic techniques to separate the protein fractions. It was found that miniature swine metabolize  $^{75}\text{Se}$  very similarly to the large breeds, so these studies and future replicates are being conducted with miniatures to aid in ease of handling and ease of blood sampling.

As a part of the intestinal carbohydrase study in sheep,  $^{14}\text{C}$  labeled glucose and cellulose were also used. The rate of glucose absorption per unit serosal area was greatest in the jejunum, least in the colon, and greater in the cecum than the ileum when glucose solutions were injected into the lumen of isolated segments. Injection of iodoacetate decreased the rate of glucose absorption in the jejunum by 50 to 66 per cent and in the ileum by 10 to 15 per cent while having no effect on glucose absorption in the cecum. Radiochemical analysis indicated that the  $^{14}\text{C}$  activity present in the blood was in the glucose fraction. Radioimpurities due to radiolysis made it impossible to evaluate the degree of cellulose utilization. The utilization of maltose in the jejunum was twice as great as in the ileum and compared well with the relative amounts of maltase present in the mucosal cells of the two areas. No starch utilization was detected during the 30 minute experimental period.

#### Discussion:

Whole-body gamma irradiation has a temporary effect on strontium and calcium metabolism with minor alteration in net retention, so this approach is being replaced with emphasis on other phases. Likewise, research on  $^{144}\text{Ce}$ - $^{144}\text{Pr}$  will continue only as long as we see changes in blood platelet and white cells in the animals now on experiment.

Since we have definitely established differences in <sup>99</sup>Mo metabolism due to species, rations, and route of administration, we expect to expand these investigations to explain these differences. This will include both chemical and radiochemical separation of components of gastrointestinal contents, and tissue and quantitation of enzyme activity. From these results, we should have more logical explanations for differences in trace mineral imbalances and metabolic processes. Radiotungsten is one of the major radioisotopes reported in debris from underground atomic tests. Chemically tungsten is in the same series with molybdenum, so comparisons will be continued to study differences and similarities between <sup>185</sup>W and <sup>99</sup>Mo. Similar techniques will be continued for <sup>75</sup>Se incorporation into blood cells and plasma in order to explain differences in <sup>75</sup>Se metabolism in swine and sheep.

Valid clinical tests for deficiencies of selenium and zinc have been developed and published. These in vitro blood cell incubations with radioisotopes will be expanded to include other ions which may be associated with irradiation and hair color.

Further investigations are needed to determine if carbohydrate enzymes may be significantly altered by ration changes, but this will not be pursued further in the near future.

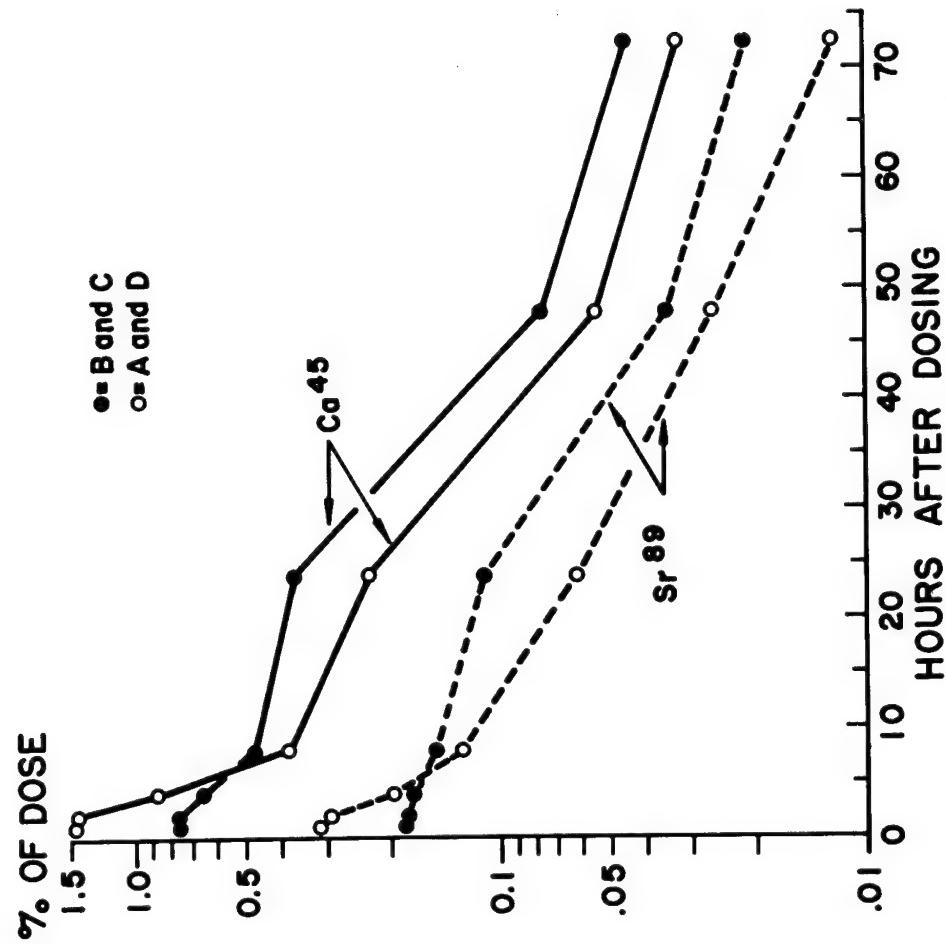


FIGURE 1. Levels of  $^{90}\text{Sr}$  and  $^{45}\text{Ca}$  per liter of blood plasma of orally dosed swine. The treatments were controls (A); dosed 1 day after irradiation (B); 3 days (C); and 7 days (D).

## Radioiodine and Dairy Cattle

J. K. Miller

### Scope:

Following a contaminating event, grazing cattle could consume considerable radioiodine because of the relatively large land area they graze. Numerous investigations with cattle have studied passage of  $^{131}\text{I}$  into cow's milk, since this is considered an important potential source of radioiodine in the human diet. Intakes of  $^{131}\text{I}$  which could produce injury to cattle greatly exceed levels that would render milk unfit for human consumption. However, milk would be acceptable for use within a few weeks after consumption of radioiodine contaminated feed by lactating cows due to rapid excretion and physical decay. It then becomes of interest to determine the effects of such exposure on later production and health of cattle.

### Status and Progress During Report Period:

Numerous investigations of iodine metabolism are available which aid in understanding the effects of  $^{131}\text{I}$  exposure on cattle. These include studies of gastrointestinal absorption and excretion, excretion in milk and urine, thyroid uptake and release, and the effects of various iodine compounds and goitrogens on these measurements. Radioiodine toxicity has been studied extensively in sheep in this country and in lactating cows in the United Kingdom. Additional investigations with mature cows and growing heifers have been conducted at this Laboratory. To date, 4 mature cows and one member of each of 9 pairs of identical twin heifers have received single doses of from 140 to 210  $\mu\text{c}$   $^{131}\text{I}/\text{kg}$  body weight. In these studies (ORO-648), tracer levels of one iodine isotope ( $^{125}\text{I}$ ) were used to measure changes in iodine metabolism resulting from thyroid damage due to large amounts of another iodine isotope ( $^{131}\text{I}$ ).

Thyroid activity 6 months after  $^{131}\text{I}$  treatment was reduced as shown by average decreases of 64 percent in plasma protein-bound iodine, 80 percent in thyroid  $^{131}\text{I}$  uptake, and 56 percent in thyroid secretion rate. Although body weights within twin heifer pairs did not differ significantly ( $P > .5$ ) at time of  $^{131}\text{I}$  dosing, heifers receiving high  $^{131}\text{I}$  levels averaged 5 percent heavier ( $P < .05$ ) than their twins 6 months later.

All heifers were bred after  $^{131}\text{I}$  dosing if they had passed 14 months of age. Four controls and one  $^{131}\text{I}$  dosed heifers are now milking. Within 6 months, six pairs of identical twin heifers will be in production. Records will be kept through at least two complete lactations to determine the effects of  $^{131}\text{I}$  induced

hypothyroidism. Three cows have each completed one lactation since thyroid irradiation and two are beginning their second lactations. In order to maintain production past the 4th month of lactation in these three cows, it has been necessary to feed iodinated casein (containing 1 percent L-thyroxine activity). A cretin calf born two weeks after one of the cows had been dosed with 100 mc  $^{131}\text{I}$  has developed almost normally after 6 months of iodinated casein feeding. This animal, a heifer, was recently bred and is to be kept through calving and subsequent lactation.

Discussion:

In order to apply information gained in these experiments to other situations, an estimate of irradiation dose to the thyroid is desirable. Thyroid dose estimates for animals in the present report ranged from 12,000 to 27,000 rads. These estimates are based on a number of factors in addition to the more precisely measured physical characteristics of  $^{131}\text{I}$ . These include fractions of the  $^{131}\text{I}$  intake going to the gland, average residence time of the  $^{131}\text{I}$ , and mass of the gland. With proper equipment, the first two can be measured with reasonable precision. The major question associated with determining the radiation dose to the thyroid is obtaining a satisfactory estimate of thyroid size in living animals. Thyroid weights of 6 cows slaughtered at this laboratory averaged 46 g with a range of 30 to 58 g. Thyroid and body mass were related ( $r = 0.85$ ,  $P < .05$ ) with an average of 100 mg thyroid tissue per kg body weight. This is much higher than the 15 g reported for cows in England and explains the low radiation dose estimates obtained at this laboratory. Thyroid and body weights were also significantly correlated for 66 calves slaughtered in previous experiments ( $r = 0.41$ ,  $P < .01$ ).

The Oak Ridge National Laboratory has been collecting six bovine thyroid glands weekly from a local slaughter house for a number of years. Weights of these glands together with additional data describing the animals from which they were taken are available for 3 years.

This information includes sex, age, breed, body weight, and type of salt (plain or iodized) fed. These data are being summarized in an effort to obtain parameters by which thyroid weights of living animals might be more accurately estimated.

Radiocerium as a Non-absorbed Reference Material for Determining  
Gastrointestinal Sites of Mineral Absorption and Excretion in  
Cattle

J. K. Miller

Scope:

Some of the absorbed minerals are excreted through the gut. An ordinary balance study does not separate such mineral material from the portion which originally failed of absorption. The re-excreted portion may be a large part of the total amount voided in the feces. Cerium-144 has been used at this Laboratory as a non-absorbed reference substance for determining net absorption or excretion in various sections of the bovine digestive tract of a number of nutrients including Ca and P (ORO-598), I (ORO-624), Zn (ORO-625), and Na, K, Mg, and N (ORO-648). This technique has an advantage over fistulas or isolated gut segments in that the experimental animal is not placed in an artificial or stressed condition.

To give valid results for this purpose, an indicator must meet at least the three following requirements: 1) it must be unabsorbed and recoverable in the feces, 2) mix with ingesta, and 3) move through the digestive tract at the same rate as ingesta. Numerous investigations of fecal excretion of chromic oxide have been reported. Investigations evaluating radiocerium, which has been shown to have a gastrointestinal distribution comparable with chromic oxide (ORO-625), as an inert reference material have recently been completed.

Status:

The series of experiments utilized a total of 38 dairy cattle ranging in age from young calves to mature cows. Daily fecal recovery of  $^{144}\text{Ce}$  from 11 animals averaged 97 percent ( $\text{CV} = 19\%$ ) of the daily dose by the 4th day, satisfying requirement 1. No pattern of diurnal variation in twice daily administered  $^{144}\text{Ce}$  excretion was evident in fecal concentrations during successive 2 hour collections. Hourly variations in  $^{144}\text{Ce}$  concentrations were slightly lower ( $\text{CV} = 11\%$ ) than corresponding  $\text{Cr}_2\text{O}_3$  concentrations ( $\text{CV} = 14\%$ ). Since excretion of dry matter and both indicators were closely related (Figure 1), much of the variation in indicator excretion was due to amount of feces obtained.

Following single doses of 10 g  $\text{Cr}_2\text{O}_3$ , 500  $\mu\text{c}$   $^{144}\text{Ce}$  adsorbed onto 190 g soybean meal, and 500  $\mu\text{c}$   $^{141}\text{Ce}$  in 60 ml  $\text{H}_2\text{O}$  to each of two cows, the first measurable amounts of any indicator appeared in samples taken 10 hours after dosing (Figure 2). Maximum excretion occurred between 20 and 40 hours after dosing. By the

36th hour, half of each indicator had been voided. Total 90 hour excretions averaged 99 percent of the Cr<sub>2</sub>O<sub>3</sub>, 95 percent of the <sup>144</sup>Ce, and 92 percent of the <sup>141</sup>Ce.

Within animal comparisons of <sup>141</sup>Ce given by capsule with <sup>144</sup>Ce previously mixed in the feed were made for both milk- and grain-fed calves. With grain fed calves, no differences were detected in gastrointestinal distributions of the two radiocerium isotopes (Figure 3), indicating the adequacy of the more simple capsule dosing method and satisfying requirement 2. However, with milk-fed calves, capsules containing <sup>141</sup>Ce went to the rumen while milk containing the <sup>144</sup>Ce was directed to the abomasum by reflex closure of the esophageal groove.

#### Discussion:

In the digestive tract contents of 19 animals, over 90 percent of the radiocerium was adsorbed onto undigested residue as shown by ultracentrifugation or availability to cation exchange resin. The firm association of cerium with residues in the digestive tract is evidence it most likely moves with ingesta, thus satisfying requirement 3. To obtain a further indication of whether cerium and solid matter move together through the digestive tract, correlations between <sup>144</sup>Ce and dry matter were calculated by segments. Highly significant positive correlation coefficients were obtained for all gastrointestinal segments of 12 animals.

Radiocerium appears to be a satisfactory indicator for determining digestibility and gastrointestinal absorption and secretion sites for nutrients associated with feed residues in ruminating animals. However, the method is of limited usefulness in the anterior portion of the digestive tract of nursing calves. Its validity in studies of soluble nutrients which are not bound to ingesta and thus may move independently has not been determined.

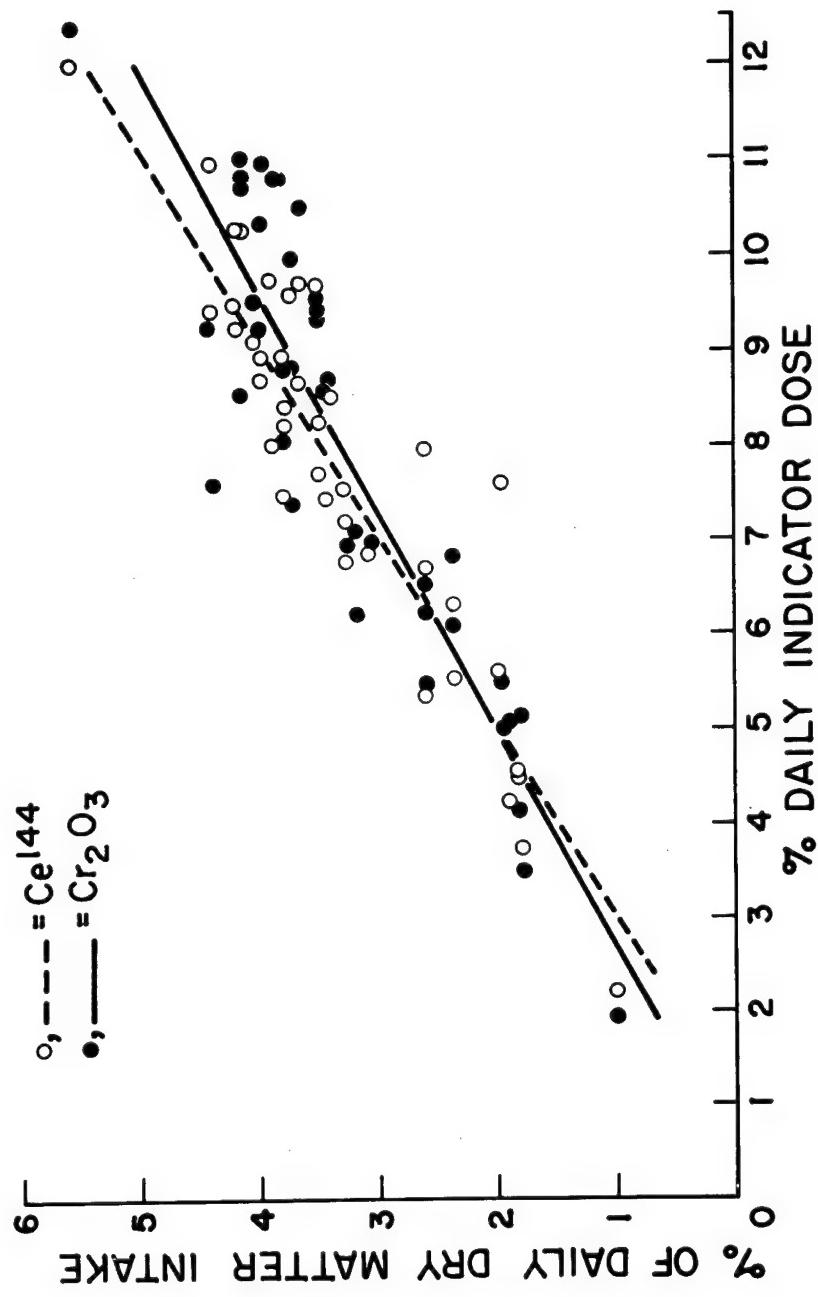


Figure 1. Relationships between fecal dry matter excretion and excretions of  $\text{Ce}^{144}$  and  $\text{Cr}_2\text{O}_3$  during twice daily dosing of two dairy cows.

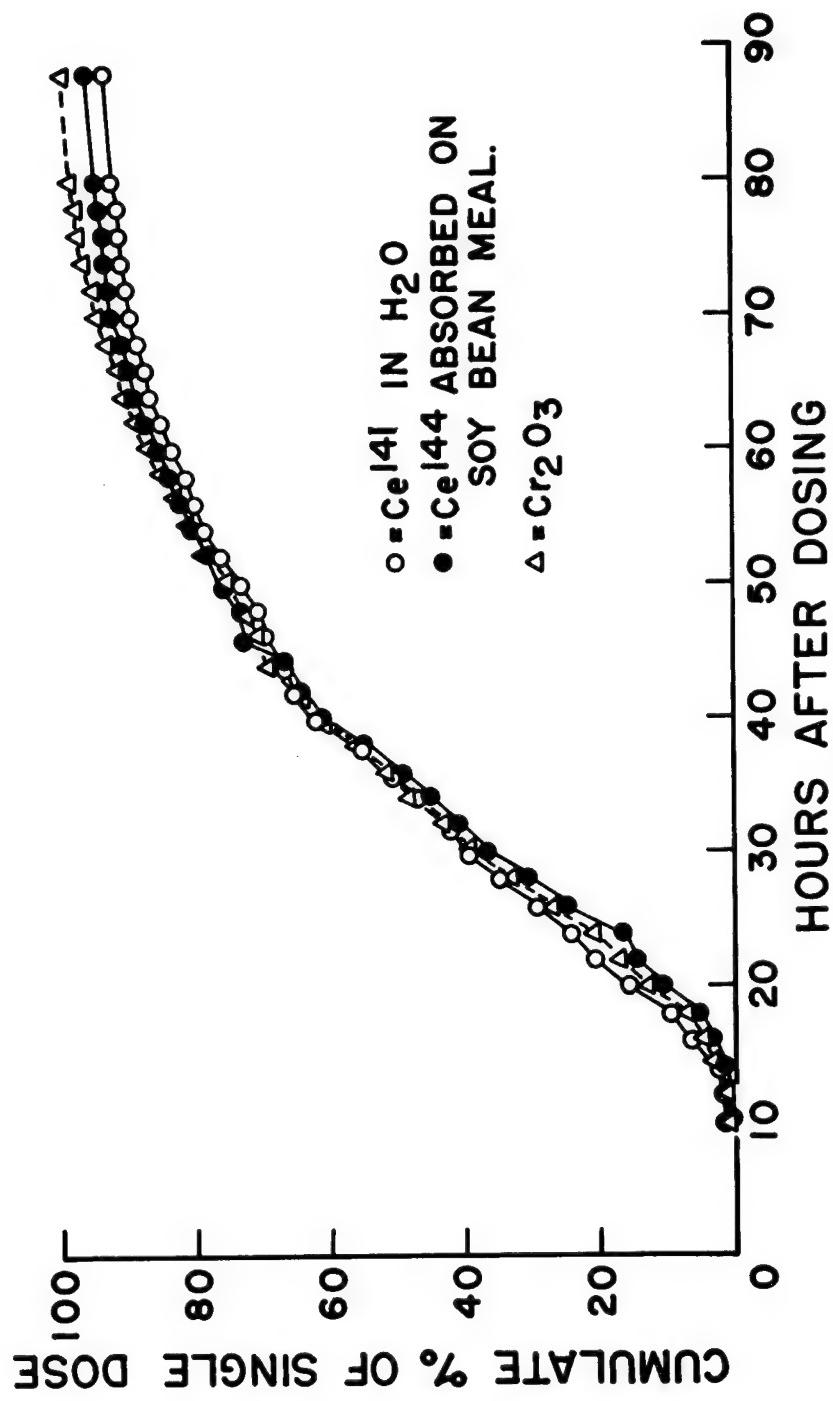


Figure 2. Cumulative excretion of single doses of  $^{141}\text{Ce}$  administered in water,  $^{144}\text{Ce}$  adsorbed onto soybean meal, and  $\text{Cr}_2\text{O}_3$  as the powder by two dairy cows.

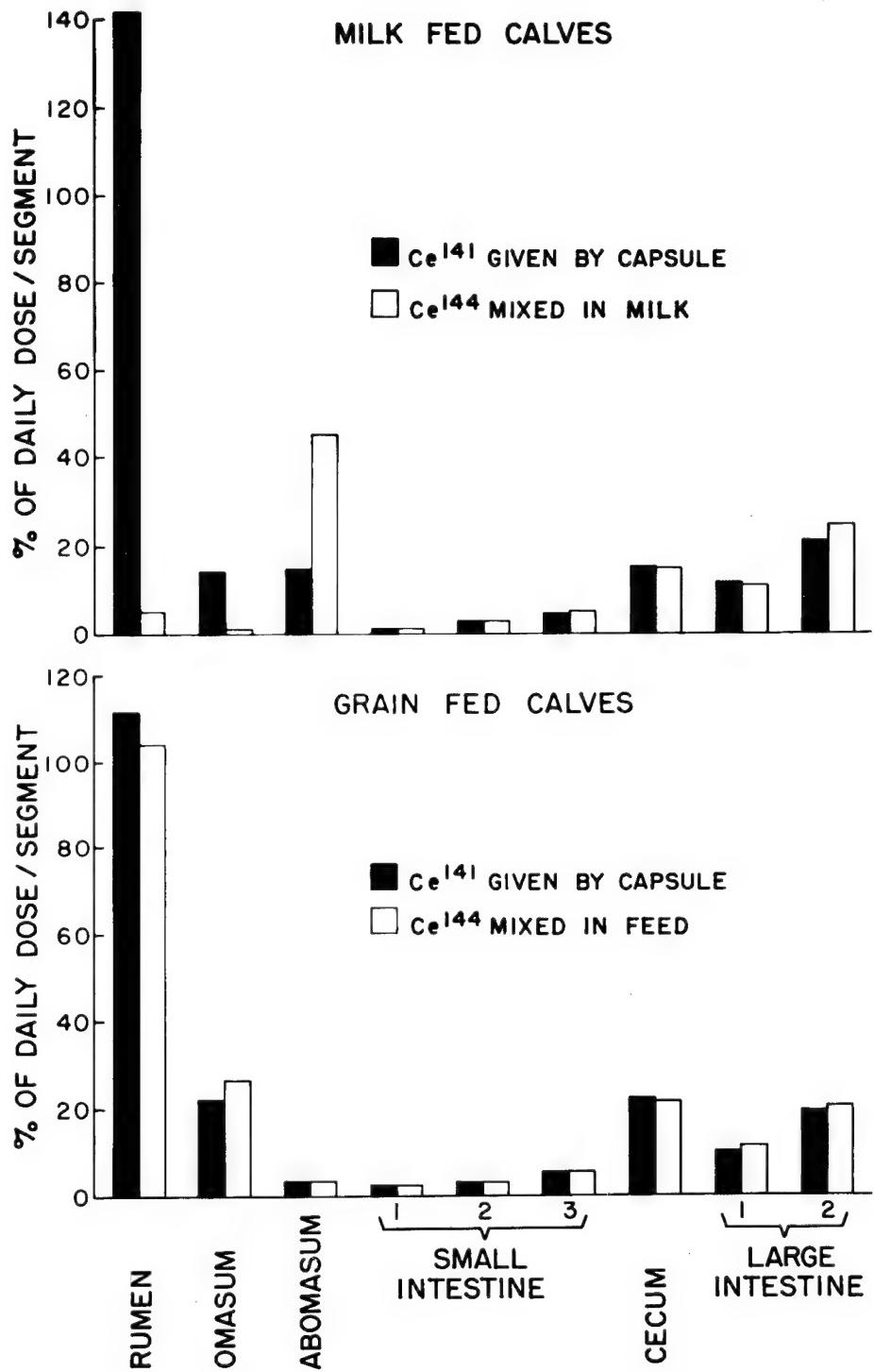


Figure 3. Comparative distributions in the calf digestive tract of  $^{141}\text{Ce}$  given by capsule and  $^{144}\text{Ce}$  mixed in grain or milk.

## Radiotoxicity in Poultry

F. R. Mraz

### Scope:

Hazards to avian species from internal emitters are the primary concern. A knowledge of the effects of whole-body irradiation from external emitters and organ and cellular irradiation from internal emitters is necessary for predicting the hazards that might be encountered in the event fission product contaminations become widespread. Further, it will form the basis for developing safeguards that may be employed for protection to counter each exposure to radiation.

### Radiostrontium ( $^{90}\text{Sr}$ - $^{90}\text{Y}$ )

Status: As low as 100  $\mu\text{c}$   $^{45}\text{Ca}$  administered at 4 or 8 days of incubation produced broken or fractured bones in chick embryos. The same level of  $^{90}\text{Sr}$ - $^{90}\text{Y}$  was less effective than  $^{45}\text{Ca}$  producing these fractures when administered at 8 days of incubation. The changes produced by  $^{45}\text{Ca}$  injected at 14 days of incubation,  $^{90}\text{Sr}$ - $^{90}\text{Y}$  at 4 or 14 days and  $^{147}\text{Pm}$  injected at 4, 8, or 14 days of incubation were developmental abnormalities frequently produced with external radiation. The administration of 50 or 100  $\mu\text{c}$   $^{90}\text{Sr}$ - $^{90}\text{Y}$  to 8-day embryos increased mortality over that experienced from the same dosages administered to 14-day embryos. However, mortality in the surviving chicks was higher from the 14-day administration. Almost all the chicks receiving 250 or more  $\mu\text{c}$   $^{90}\text{Sr}$ - $^{90}\text{Y}$  level tended to reduce testis size at 13 weeks of age.

Progress during this report period: No effect on egg production or mortality in the surviving pullets has been observed thus far over a 4-month egg production period.

Discussion: Livability and reproductive performance in these pullets for the first year of their life does not appear to be affected by the  $^{90}\text{Sr}$ - $^{90}\text{Y}$  dosage they received as embryos or young chicks providing they survived the initial mortality period. The surviving pullets are being maintained for the coming year and those dying will be examined for any bone lesions indicating radiation damage.

### Radioiodine ( $^{131}\text{I}$ )

Status: Lower dosage than those reported in the literature have been found to produce lingering damage to the embryonic thyroid and the 0, 1 and 2-week chick thyroid. Embryonic requirements for thyroxine appear to be small until the 18th to 20th day of incubation, since most of the embryos receiving 100 or more  $\mu\text{c}$   $^{131}\text{I}$

grow to this stage and then stop development (don't draw the yolk sac into their body) even though they live as long as 29 days after initiation of incubation. As low as 2  $\mu$ c  $^{131}\text{I}$  given to a 16-day embryo reduced  $^{131}\text{I}$  uptake by the 4-week-old bird, while it took 50  $\mu$ c to reduce growth rate. The 2  $\mu$ c  $^{131}\text{I}$  chicks, however, recovered their capacity to absorb  $^{131}\text{I}$  by 6 weeks of age. The 5  $\mu$ c  $^{131}\text{I}$  chicks showed permanent partial damage to the thyroid, while those that received 50  $\mu$ c showed no appreciable uptake by the thyroid even at 15 weeks of age. Enough  $^{131}\text{I}$  has been shown to be transferred from the hen to the egg to produce the embryonic damage. Preliminary studies with small numbers of pullets remaining on experiment seem to indicate that egg production was reduced at the 5  $\mu$ c level and totally absent on the 50  $\mu$ c level.

Progress During this Report Period: Larger numbers of Pullets (at least 20 per group) have been reared from embryos given 1, 2, 5, 25, or 50  $\mu$ c  $^{131}\text{I}$  at 16 days of incubation to study the effects of these levels on reproductive performance. Examination of the cockerels remaining at 6 and 15 weeks of age have revealed that the 5  $\mu$ c level and higher of  $^{131}\text{I}$  reduced  $^{131}\text{I}$  uptake similarly to that reported. While body weights were reduced at the 25 or 50  $\mu$ c level, the testes weights per gram of body weight were not affected.

The biological half-life of  $^{131}\text{I}$  administered at 1 day of age was about 1.4 days while that of iodine was 1.6 days.

Discussion: It would appear that even in those chicks which produce enough thyroxine to hatch after exposure to  $^{131}\text{I}$  the thyroid could be damaged sufficiently to reduce growth. The pullets will be maintained for at least 1 year of egg production to ascertain if  $^{131}\text{I}$  damage to the thyroid which is not perceivable will affect egg production.

#### The Metabolism of Fission Products and Related Nuclides in Poultry

F. R. Mraz

##### Scope:

The principal objective is the study of the metabolism of fission products with special reference to their role in the metabolic pathways and to ascertain their interaction with similar nuclides. The mechanisms of discrimination between minerals at the interfaces of organs, cells and cellular particulates during the vital life processes of absorption, excretion, anabolism, catabolism, and reproduction will be investigated. The influence of dietary additives on the fission product elements is studied to ascertain the possi-

bilities of influencing their metabolic pathways. The information obtained in these studies is useful in understanding the relationship of fission products to essential minerals and thereby developing countermeasures to fission produce contaminations.

#### Radiostrontrium ( $^{89}\text{Sr}$ )

Status: The uptake of administered  $^{89}\text{Sr}$  by the embryonic bone was found to be very dynamic, entering and leaving it during incubation. The biological half-life of Sr from 14 to 70 of age days was about 20 days with only 0.4% of the  $^{89}\text{Sr}$  being found in the tibia by 70 days of age.

Progress During this report period: While the data previously obtained on  $^{89}\text{Sr}$  deposition in the tibia of embryos dosed at 8 or 14 days of incubation was quite erratic, that obtained from dosing at 4 or 8 days of incubation showed a steady rise in  $^{45}\text{Ca}$  and  $^{89}\text{Sr}$  in the tibia and femur until after the 3rd day after hatching. Slightly more than 7% of the administered  $^{89}\text{Sr}$  was found in the tibia of 3-day-old chicks dosed at 8 days of incubation while only slightly over 3% of the  $^{45}\text{Ca}$  administered at the same time was found in the tibia. More  $^{45}\text{Ca}$  was taken up by the bone than  $^{89}\text{Sr}$  initially, but at 21 days of incubation the reverse is true and remains so for the first week of growth. By the second week, the rate of  $^{89}\text{Sr}$  removed from the bone is faster than  $^{45}\text{Ca}$  and  $^{45}\text{Ca}$  recovers its lead. Comparisons of the epiphseal areas with bone shaft show higher specific activities of  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  to stable calcium in the epiphyseal portions. Autoradiographs also show that constant remodeling of the bone occurs. This explains the observation that the center of the bone shaft has not appreciably more activity in it after the embryo is dosed before 12 days of incubation than does the rest of the bone at hatching even though damage to the bone cells in this area is evident.  $\text{Sr}^{89}$  appears to be removed from the bone more rapidly than does  $^{45}\text{Ca}$ . The higher  $^{45}\text{Ca}$  and  $^{89}\text{Sr}$  counts in the tibia of chicks at or a few days after hatching than were observed during incubation indicate that the radionuclides are tied up in the yolk and then begin to be available to the chick as it draws upon the yolk for energy increasing the total  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  in the skeleton.

Discussion: Comparisons are being made of strontium uptake and biological half-life in the growing chicken with *Coturnix quail* and bob-white quail. This will permit a comparison among species that start egg production at 6 weeks, 5 months and one year of age. Calcium uptake and biological half-life at various ages will be studied simultaneously. This work will be related to the gastrointestinal and cellular component studies described in the following research proposal.

### Ca-P Relations

Status and Progress During This Report Period: Many investigators have shown that calcium requirements for birds are higher as they approach or begin egg production. Work at our laboratory as well as elsewhere has indicated that birds could adapt to different Ca and P intakes given prior to egg production, so much so, that these would influence their ability to adapt to rapid changes in dietary calcium. Concurrent increases in parathyroid gland weights with increasing stable Ca intake were accompanied by decreasing alkaline phosphatase activity suggesting a dietary Ca-parathyroid function interaction which appears to be directly related to the differences observed in  $^{45}\text{Ca}$ ,  $^{89}\text{Sr}$  and  $^{32}\text{P}$  uptake from the gastrointestinal tract. When females from each of the dietary and adaptation periods treatment groups (9-19 weeks; 10-19 weeks and 15-19 weeks) were placed on an intermediate (2.0%) dietary stable calcium level for one week prior to oral dosing, the  $^{45}\text{Ca}$ ,  $^{89}\text{Sr}$  and  $^{32}\text{P}$  tibia retention pattern was markedly altered by the length of adaptation and the direction of the stable Ca shift (0.65% to 2% or 2.6% to 2.0%). Similarly shifts in parathyroid gland weight (and presumably function), plasma alkaline phosphatase and plasma calcium were also related to the dietary stable Ca changes, again indicating an interaction between parathyroid gland function and absorptive capacity which was affected by the length of the adaptive period. The  $^{89}\text{Sr}/^{45}\text{Ca}$  uptake ratios were not affected by previous calcium intake regardless of the length of the adaptation period in growing pullets. The perfusion of the intestinal tract of growing chicks *in vivo* with levels of calcium 0, 1, 5, or 25 mM for 100 minutes at a flow of 1 ml/minute confirmed the lack of change in the  $^{89}\text{Sr}/^{45}\text{Ca}$  ratio, even though both the percent of dose of  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  taken up as Ca increased dropped (Table 1). The same levels of strontium, however, reduced the ratio of  $^{89}\text{Sr}/^{45}\text{Ca}$  while reducing  $^{89}\text{Sr}/^{45}\text{Ca}$  uptake to a lesser degree than calcium. These data are on  $^{89}\text{Sr}/^{45}\text{Ca}$  ratios and are opposite to those observed with growing rats (Mraz 1962, Proc. Soc. Exptl. Biol. Med. 110:273) where the ratio of  $^{85}\text{Sr}/^{45}\text{Ca}$  rose with Ca increases while it remained the same with Sr increases. Preliminary data with *Coturnix* quail (Table 2) seem to indicate that they follow more closely the trend in Sr/Ca ratios observed with growing rats, where the ratio of  $^{85}\text{Sr}/^{45}\text{Ca}$  rose with Ca increases remaining virtually the same with Sr increases, than chickens. In rats (Mraz 1962, Proc. Soc. Exptl. Biol. Med. 111:429) the uptake of  $^{85}\text{Sr}$  by mitochondria from kidney and liver was reduced to a greater degree by added calcium than was the uptake of  $^{45}\text{Ca}$ . Uptake of  $^{85}\text{Sr}$  was increased to a greater degree than was the uptake of  $^{45}\text{Ca}$  by addition of Sr. Combinations of the two gave results comparable to that with Sr alone.

Discussion: In view of the large "Cage Layer Fatigue" problem in the poultry industry as well as other problems in laying pullets in which Ca metabolism is involved, a knowledge of the importance of the prelaying diet upon Ca metabolism and mechanisms is important.

It is hoped to ascertain the immediate course of the increased Ca uptake and adaptation to diets by pullets nearing or in egg production through an examination of the uptake of Ca by the cellular components. The secret of the differences observed in Sr/Ca ratios may also be there.

The initial experiments will consist of the perfusion of growing chicks, sexually mature cockerels and pullets of both chickens and Japanese quail with solutions containing <sup>45</sup>Ca and <sup>89</sup>Sr and varying in stable calcium and strontium. The uptake of <sup>45</sup>Ca and <sup>89</sup>Sr will be studied and then the cellular components of the liver and gut mucosa will be examined for <sup>45</sup>Ca and <sup>89</sup>Sr content to see if any correlation particularly in uptake and ratios exist.

Removal of mitochondria and microsomes from the liver and intestinal mucosa of birds on diets varying in Ca and Sr and a study of their uptake of <sup>45</sup>Ca and <sup>89</sup>Sr at different levels of calcium and strontium in the incubation media will be made to see if it relates to in vivo uptake by birds on similar diets being perfused as above.

A study of the adaptation by the bird to Ca and Sr feeding for different length periods with or without changes in these elements prior to <sup>45</sup>Ca and <sup>89</sup>Sr administration will also be made.

Finally, a series of experiments will be conducted to ascertain what factors other than calcium and strontium influence mitochondrial and microsomal uptake of <sup>45</sup>Ca and <sup>89</sup>Sr in vivo.

TABLE 1. Absorption of  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  From Perfused Small Intestines of Chicks as Affected by Ca, Sr, and Mg

Alkaline Earth in Perfusionate	$\mu\text{M}$	% of Total Dose		Ratio $^{89}\text{Sr}/^{45}\text{Ca}$
		$^{89}\text{Sr}$	$^{45}\text{Ca}$	
Ca	0	16.9	30.3	0.56
	1	10.9	24.1	0.45
	5	5.6	9.9	0.57
	25	2.7	5.4	0.50
Sr	0	16.9	30.3	0.56
	1	10.2	23.5	0.43
	5	7.2	22.4	0.32
	25	5.4	18.2	0.30
Ca	5)			
	)	4.7	12.3	0.38
Sr	25)			
Mg	0	16.9	30.3	0.56
	1	7.2	21.6	0.33
	5	4.2	17.3	0.24
	25	5.7	19.7	0.29

TABLE 2. Absorption of  $^{89}\text{Sr}$  and  $^{45}\text{Ca}$  From Perfused Small Intestines of Adult *Coturnix Quail* as Affected by Ca and Sr

Alkaline earth	$\mu\text{M}$	Sex	% of Total Dose		Ratio $^{89}\text{Sr}/^{45}\text{Ca}$
			$^{89}\text{Sr}$	$^{45}\text{Ca}$	
Ca	0	M	3.3	10.5	0.31
		F	4.0	15.7	0.26
Ca	5	M	0.6	1.1	0.51
		F	1.1	2.1	0.52
Sr	5	M	0.9	3.5	0.26
		F	1.5	7.1	0.21
Ca	2.5)	M	0.7	1.5	0.47
Sr	2.5)	F	4.8	16.1	0.30

## SOIL CHEMISTRY

The entrance of fission products into the food chain requires increased attention as larger quantities of radioactive materials are released into man's environment. When fission products are incorporated into the soil, they become available to plants through normal ion uptake processes. The objective of the soil chemistry research program is to find a means of predicting the availability of these materials. Therefore, the various aspects of the program consist of studying retention and rates of distribution of isotopes in the soil.

### Properties of Clay Systems Controlling Ion Exchange Kinetics and Ion Exchange Equilibrium

R. G. Gast

#### Scope:

The soil chemistry research program is concerned with studying soil properties affecting cation availability to plants. This would then apply to nutrient cations and those fission products existing in the soil as cations. Under conditions where this availability is controlled by diffusion processes it then becomes a problem of studying the soil properties controlling these processes and the rate laws which are involved. The report here deals with the former, i.e., the soil properties controlling cation diffusion rates.

Earlier results consistently pointed to a close relationship between the clay-cation interaction and cation mobilities or diffusion rates. On this basis investigations were conducted to test for the existence of a quantitative relationship between this interaction, which can be reflected by an activity coefficient, and diffusion rates. Such a relationship was found for Na ion in bentonite-water systems.

At this point there was a choice between 1) attempting to develop and utilize these concepts of a single ion activity coefficient for studying the soil properties controlling diffusion rates or 2) turning to a more rigorous thermodynamics approach to determine the factors reflected in the deviations from ideal behavior as indicated by the activity coefficients. While there seems to be a consistent and close relationship between the single ion activity coefficients, unfortunately, the non-thermodynamic assumptions necessary in arriving at these values make further interpretation impossible. Therefore, it was decided to turn to the more rigorous approach through the thermodynamics of ion exchange.

Status:

One of the first concepts being tested is that of the hydration status of the cation in relation to its behavior in both ion exchange equilibrium and kinetics. One explanation for the relative mobilities observed for monovalent cations is that varying proportions of different cations are dehydrated due to the interaction with the clay, and hence essentially immobilized. Similarly, it has been postulated that selectivity sequences for monovalent cations are controlled by the relative free energies of interaction vs. the free energies of hydration. That is, if the free energy of interaction is greater than the free energies of hydration, the cations will be dehydrated and selectivity will follow the order of decreasing crystal radii or ( $\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$ ). If the converse is true, selectivity will follow the order of decreasing hydrated radii or ( $\text{Cs} > \text{Rb} > \text{K} > \text{Na} > \text{Li}$ ). The hydration energies are fixed, and known. The energies of interaction can be varied by varying the anion field strength of the exchange site. The exchange sites of a strong acid exchanger generally have low anionic field strengths and hence the free energies of interaction of cations with water will be greater than with the exchanger. Thus, cations remain hydrated and selectivity follows the order of hydrated radii. In contrast, the exchange sites of a weak acid exchanger have high ionic field strengths and hence the free energies of interaction of cations will be less than with the exchanger. The cations are then dehydrated and follow the order of crystal radii.

Applying this reasoning to exchangers having mixed functional groups, it would be expected that as the pH of the system is increased, the rates of weak to strong acid sites would increase; that is, at a low pH the exchanger would behave typically as a strong acid type and at a high pH it would behave as a mixed weak and strong acid type. At low pH then, selectivity should follow the order of hydrated radii. At a high pH the weak acid groups would also be functional giving a selectivity sequence following the crystal radii sequence. In this case, the observed selectivity sequence would be a result of the relative proportions of the two functional groups.

Clays in general, and kaolinite in particular, are known to be polyfunctional in nature. Investigations have then been designed to test the concepts outlined above by studying the pH dependence of the selectivity coefficients for the alkali metal cations on kaolinite and Wyoming bentonite. The experimental design is as follows:

The clay gels were placed in dialysis bags and suspended in solutions containing  $\text{CsCl}$  and  $\text{NaCl}$  with  $\text{Cs:Na}$  ratios of 9:1, 5:5, and 1:9 and a total salt concentration of  $1 \times 10^{-3}$  N. The external solutions were adjusted to three pH levels, 3.5, 5-6, and 8+.

The solution at pH 3.5 was prepared by adding HCl, that at pH 5-6 by using distilled water and that at pH 8+ by adding  $1 \times 10^{-4}$  N NaOH. External solutions were renewed until equilibrium was established, as indicated by no further change in Cs or Na concentrations. Equilibrium times exceeded three weeks in all cases. The dialysis bags were then opened and samples taken for Cs and Na determination of the selectivity coefficients. The Na and Cs concentrations on the exchanger were determined by extraction with 1 N ammonium acetate and subsequent analysis. Five samples were extracted and analyzed from each dialysis bag.

Progress During This Report Period:

Results of two typical analyses are given in Table 1 indicating the precision attainable by this technique. Complete results giving the Na and Cs saturation and selectivity coefficients are given in Tables 2 and 3. Selectivity coefficients have not been corrected for inclusion of free electrolyte in the exchanger phase. The maximum correction necessary for the bentonite (assuming no negative adsorption) would be about 2%.

Negative adsorption measurements have been made using this bentonite at four clay concentrations, and over an equilibrium NaI concentration ranging from  $1 \times 10^{-3}$  to  $10 \times 10^{-3}$  moles per liter. Results from the 5% clay are presented graphically in Figure 1, plotted as concentration of the external solution vs. the average concentration in the pore space of the gel. These results indicate the correction due to electrolyte inclusion would be about 1.3%. Corrections will be somewhat larger in the case of kaolinite (a maximum of about 5%), but negative adsorption data are not yet available. These corrections will be made for both clays before final interpretation of the data.

Discussion:

These data must be considered as preliminary and hence can only be evaluated qualitatively at this time. However, the following two general observations can be made.

1) The selectivity coefficient, and hence relative affinity for Cs over Na, does increase (i.e., a value greater than one indicates preference of the clay for Cs over Na) with decreasing Cs saturation for both clays and all pH's. This is a fairly general observation for most exchangers and is generally considered as resulting from their having exchange sites with a range of effective field strengths.

2) Kaolinite is different from bentonite in that the selectivity coefficient is very pH dependent.

The absence of any pH dependence in the case of bentonite indicates very little weak acid character. The large pH dependence of kaolinite selectivity coefficients is not surprising, but it appears to be in the opposite direction from that expected. Using the above reasoning one would expect the relative selectivity for Cs over Na to decrease with increasing pH rather than increase as it did. These results suggest that the anionic field strength did increase on increasing pH. But the increase was not great enough to dehydrate the cations which would have resulted in a selectivity decrease or perhaps even reversal.

In order to further check these concepts, these data are being extended to include Cs:Na ratios of 7:3 and 3:7 and to include selectivity data for the cations Li, Rb, and K to Na. Calorimetric determinations of heats of exchange will also be investigated, which should help elucidate the factors involved when coupled with the free energies of exchange available from the selectivity data.

TABLE 1. Typical Results of Clay Analysis Used in Calculating Equilibrium Selectivity Coefficients

Me Na per gm clay	Me Cs per gm clay	Me (Cs + Na) per gm clay	Cs/Na on clay	$K_s^C$ Na
<u>5% Bentonite Cs:Na = 9:1 pH 8+</u>				
0.056	0.866	0.922	15.45	1.72
0.052	0.848	0.900	16.25	1.81
0.053	0.878	0.931	16.51	1.83
0.051	0.847	0.898	16.68	1.85
0.052	0.878	0.930	17.04	1.89
Av. 0.053	0.863	0.916	16.39	1.82
<u>50% Kaolinite Cs:Na = 5:5 pH 8+</u>				
0.00196	0.0153	.0173	7.82	7.82
0.00190	0.0151	.0170	7.94	7.94
0.00190	0.0150	.0169	7.90	7.90
0.00188	0.0153	.0172	8.13	8.13
0.00191	0.0149	.0168	7.79	7.79
Av. 0.00191	0.0151	.0170	7.92	7.92

TABLE 2. Na and Cs Saturation and Equilibrium Selectivity Coefficients for 5% Wyoming Bentonite Clay Gel

pH of external solution	Me Na per gm clay	Me Cs per gm clay	Me (Cs + Na) gm clay	Cs K Na
Cs:Na in solution = 9 : 1				
3.5	0.0413	0.5634	0.6047	1.51 ± .10
5-6	0.0455	0.7648	0.8104	1.91 ± .12
8 +	0.0527	0.8633	0.9160	1.82 ± .07
Cs:Na in solution = 5 : 5				
3.5	0.1167	0.4214	0.5381	3.16 ± .12
5-6	0.1564	0.6066	0.7630	3.88 ± .29
8 +	0.1976	0.6917	0.8894	3.50 ± .09
Cs:Na in solution = 1 : 9				
3.5	0.2958	0.2041	0.5000	6.21 ± .28
5-6	0.3603	0.2915	0.6519	7.31 ± .53
8 +	0.5926	0.3427	0.9352	5.21 ± .15

TABLE 3. Na and Cs Saturation and Equilibrium Selectivity Coefficients for 50% Kaolinite Clay Gel

pH of external solution	Me Na per gm clay	Me Cs per gm clay	Me (Cs + Na) gm clay	Cs K Na
Cs:Na in solution = 9 : 1				
3.5	0.000680	0.00612	0.00679	1.04 ± .13
5-6	0.000124	0.01180	0.01305	1.10 ± .17
8 +	0.000790	0.01763	0.01842	2.45 ± .28
Cs:Na in solution = 5 : 5				
3.5	0.00121	0.00422	0.00543	3.49 ± .25
5-6	0.00167	0.00918	0.01085	5.48 ± .24
8 +	0.00191	0.01510	0.01701	7.91 ± .12
Cs:Na in solution = 1 : 9				
3.5	0.00157	0.00111	0.00268	6.38 ± .60
5-6	0.00288	0.00439	0.00728	13.71 ± .52
8 +	0.00448	0.00796	0.00124	15.99 ± .34

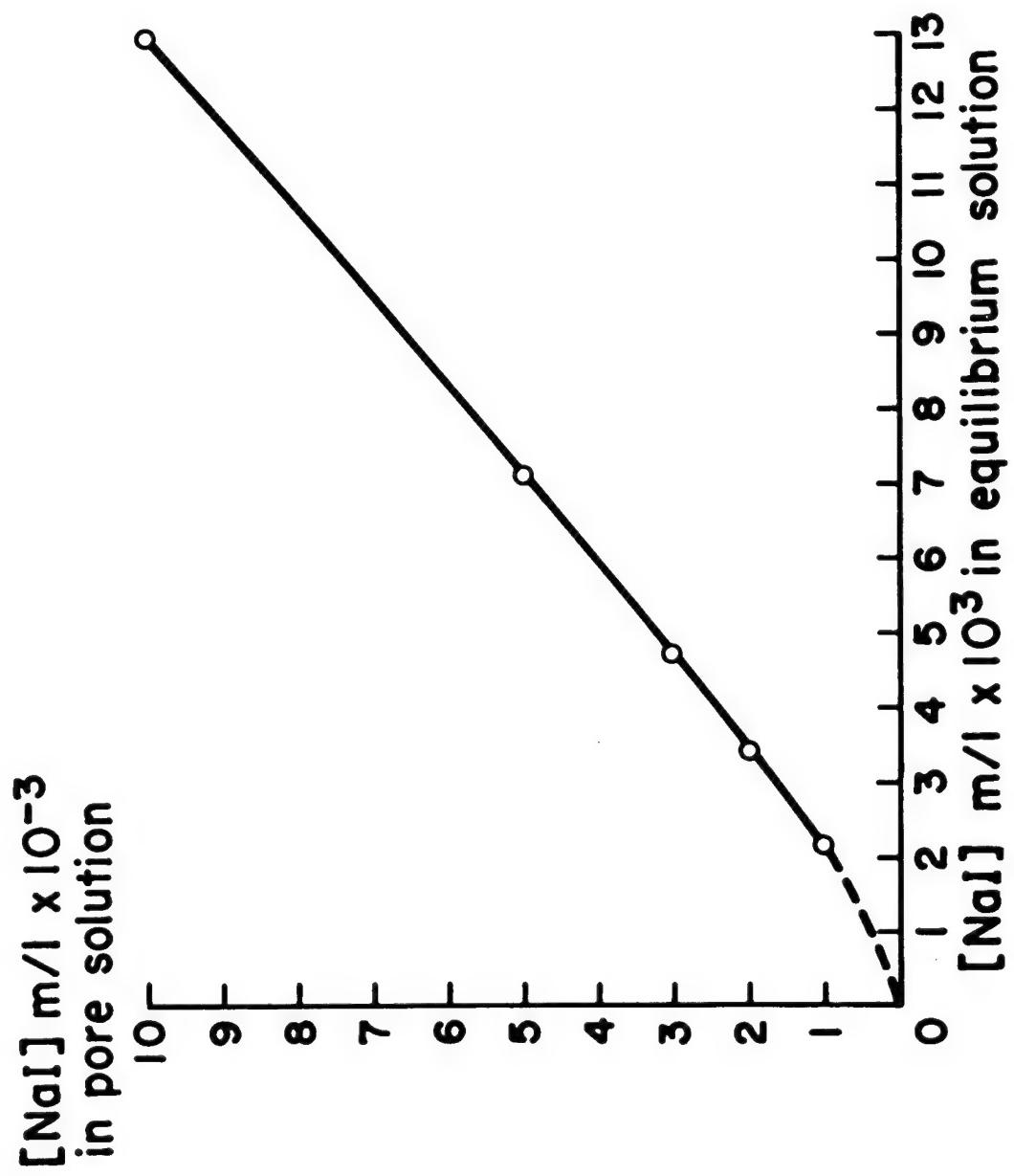


FIGURE 1. NaCl concentration in external equilibrium solution vs. the average NaCl concentration in the pore solution of a 5% Wyoming bentonite gel.

## Diffusion in Non-Idealized Systems

Gary E. Spalding

### Scope:

Diffusion is a phenomenon which, under many conditions, plays an important role in the movement of fission products through soils and their subsequent uptake by plants.

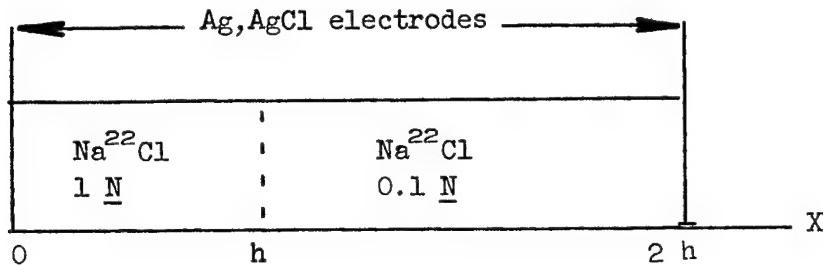
In very non-ideal systems, especially those, such as soils, which contain ion-exchanger materials, diffusion is a very complex phenomenon. The results of laboratory studies are often very difficult to interpret quantitatively and sometimes qualitatively. The present study is concerned with increasing our understanding of diffusion in these systems; it is concerned with developing adequate theoretical and experimental means of assessing the role of diffusion in the transport of fission products through soils.

### Status:

A diffusion theory which in many respects is similar to the Nernst-Planck theory has been under study for the past year. The principal new feature of the theory is that it serves to predict the rate of decay of the diffusion potential in closed-system, transient state diffusion. The equation governing the decay of the diffusion potential was initially a postulate based on empirical observations. It has recently been completely derived for all single electrolyte systems, but remains only a postulate for other systems. A potentiometric technique has been used to test the theory in single electrolyte systems, mixed electrolyte systems and ion-exchange resin membranes. The results of these experiments have been in excellent agreement with the theory. The electrolyte systems have been studied because they are experimentally simple to manipulate and they exhibit diffusion potential properties similar to those of more complex systems.

### Progress during Report Period:

While potentiometric results are encouraging, they are not sufficient to conclusively test the theoretical treatment. Also needed are data on fluxes of material in our diffusion cells. Such data have now been obtained for the cell:



In these experiments, the potentiometric data and theory were used to predict the percentage of total  $\text{Na}^{22}$  which should be in the right-hand half-cell after diffusion had occurred for a given time. The cell was then split and the half-cells were counted in a large-well scintillation crystal. The results of a series of experiments are given below.

<u>t</u> (minutes)	Percentage of total $\text{Na}^{22}$ which is in the right half-cell	
	<u>Predicted</u>	<u>Observed</u>
19.64	26.5	25.8
27.32	28.7	28.5
36.69	31.1	32.1
41.47	34.2	34.3
59.27	37.6	38.0
83.81	41.1	42.1

The agreement between predicted and observed values is within 3% error in all cases. Since the data do not indicate any systematic deviations, it is possible that refining our experimental technique might make the deviations between predicted and observed values smaller.

#### Discussion:

We have yet to obtain flux data from cells made up of resin membranes. Such information is needed to assess the usefulness of the theoretical treatment in more complex systems.

There are many questions raised by the success of the theory to describe potentiometric decay curves in systems for which we have been unable to derive the postulated equations. Our future work must follow two avenues. One of these is to obtain flux data to confirm or deny the applicability of the theory in the more complex systems. The other is to work with simpler systems (but of a complexity greater than those systems for which the theory has been derived) where we may observe diffusion potentials without electrode potentials being appreciable.

### Specific Reactions Affecting Cation Availability in Clay Minerals

W. D. Klobe

#### Scope:

The present research program was initiated to provide data for establishing the significance of fixation reactions in controlling cation availability to plants. Cs and Sr are among the more important fission products which occur in the soil as cations, and are associated with the exchange complex or clay fraction of the soil. Isotopes of these two elements, <sup>134</sup>Ca and <sup>85</sup>Sr, are being used in nearly all of the exchange reaction studies, due to their longer half-lives. The more homogeneous pure clay minerals are being used in the initial experiments in order to provide simpler and better defined systems for determining the conditions under which these reactions occur.

It has been assumed that the availability of fission products for entrance into the food chain is governed largely by the rate at which they move through the soil to root surfaces. The amount of fixation, or degree of cation retention, within the lattice structure of various clay minerals should have a direct effect on the system concentration and consequently on the availability to plant roots. The term fixation is herein defined as the ability of a clay to retain cations in a state such that they are not available for rapid isotopic exchange in a rapidly stirred solution.

#### Status:

Previous investigations of Cs sorption and desorption reactions with 40-20 mesh Montana vermiculite indicated Cs availability was related to the extent of collapse of the mineral lattice. This is evident from the slow reaction rates obtained in

the presence of one symmetry of stable CsCl. The Sr-vermiculite reactions differed in that the Sr ion was readily exchangeable under all the conditions studied.

Progress During Report Period:

In all of the experiments described in this report, a batch technique was employed, eliminating flow rates in the experimental system from being a rate-limiting factor. In most cases, a double 0.10  $\mu$  Ga-9 Gelman filter was used in procuring one ml. samples of solution for gamma radioassay. Samples were returned to the reservoir after each activity measurement.

Studies were made to determine if the fixation properties exhibited by 40-20 mesh vermiculite would be as readily apparent in the smaller size fractions. Sorption and desorption rates of  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  were determined using < 120 mesh, 0.2-2.0  $\mu$ , and < 0.2  $\mu$  size fractions of vermiculite where the initial labels were (a) prior to and (b) after the addition of one symmetry stable salt. The reactions were again specific for Cs in all size fractions, and the rate of isotopic exchange was greatly reduced after one symmetry of stable CsCl was added to the solution (see figure 1). The amount of fixation is depicted by the difference in the Q/Q<sub>0</sub> equilibrium values of the first and second aliquots of  $^{134}\text{Cs}$ , where the aliquots were added prior to and after the addition of one symmetry stable CsCl, respectively. There was also a noticeable difference in the Q/Q<sub>0</sub> equilibrium values where the vermiculite was equilibrated with one symmetry of stable CsCl prior to labeling, compared to where it was labeled essentially electrolyte free prior to adding the stable salt. This gives evidence of a possible lattice collapse which would inhibit the sorption of  $^{134}\text{Cs}$  in the former case, while in the latter situation, the sorbed isotope would be trapped in the intralattice areas and would become unavailable for rapid isotopic exchange. In contrast to the Cs retention, Sr was rapidly exchangeable in all cases.

Cs and Sr exchange reaction rate determinations were also made using 2-50  $\mu$  Georgia kaolinite, < 0.2  $\mu$  Wyoming bentonite, and < 150 mesh Fithian illite to determine whether the fixation phenomenon was restricted to vermiculite or mica-like materials. Fixation did occur in kaolinite and bentonite after the addition of one symmetry stable CsCl, but to much lesser extent than in vermiculite, and may have resulted from impurities in the clays. The degree of Cs fixation in illite was not as great as in vermiculite, but was substantially more than that which occurred in kaolinite and bentonite. Sr was again rapidly exchangeable in all cases.

Additional fixation studies were made using the 0.2-2.0  $\mu$  and < 0.2  $\mu$  size fractions of vermiculite to determine whether the fixation is due to a collapse of the lattice by CsCl which should

result in a trapping of all cations present in the intraparticle region. The samples were labeled with  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  prior to the addition of one symmetry stable  $\text{SrCl}_2$  and  $\text{CsCl}$ , respectively; i.e.,  $^{134}\text{Cs}$  in the presence of  $\text{SrCl}_2$  and  $^{85}\text{Sr}$  in the presence of  $\text{CsCl}$ . Upon the addition of a second aliquot of isotope there appeared to be rapid isotopic equilibrium in each experiment. These results are depicted in figure 5. There was no apparent fixation of Cs in the presence of  $\text{SrCl}_2$  but the test in this case is not conclusive. Even though the sorbed  $^{134}\text{Cs}$  was not readily released upon the addition of one symmetry stable  $\text{SrCl}_2$ , this could be due to a high selectivity of the vermiculite for Cs. The test is conclusive for Sr and any lattice collapse induced by stable  $\text{CsCl}$  did not inhibit the  $^{85}\text{Sr}$  exchange reactions.

#### Discussion:

The results may be summarized by saying that  $^{134}\text{Cs}$  is fixed (according to the definition given at the start of this report) in vermiculite size fractions ranging from 40-20 mesh through  $< 0.2 \mu$ , and in  $< 150$  mesh illite upon addition of one symmetry stable  $\text{CsCl}$  to the  $^{134}\text{Cs}$  labeling solution. Fixation also occurred in the bentonite and kaolinite clays used, but the fixation capacity was limited and may have resulted from impurities in the clays. Where no  $\text{CsCl}$  was added, or if  $\text{SrCl}_2$  was added in place of  $\text{CsCl}$ , it was not possible to distinguish whether the  $^{134}\text{Cs}$  retention was controlled by fixation, or by a high selectivity coefficient of Cs over the competing cation. In contrast to the Cs retention, Sr was rapidly exchangeable in all cases, with no evidence of fixation by any of the clays in the presence of either  $\text{CsCl}$  or  $\text{SrCl}_2$ .

It has definitely been established that fixation reactions influence cation availability. However, the amount of influence will depend upon the degree of cation retention, and will vary with different soil systems. The fixation studies described in this report were made to determine rates of cation availability in various pure clay minerals, and is an initial step in establishing the significance of these reactions in the more heterogeneous soil systems. If the exchange characteristics exhibited by soils are consistent with the reactions observed in pure clay minerals, a comparison of depletion and release rate values should indicate whether the fixation reactions are "rate-limiting" in supplying cations for plant uptake.

X-ray diffraction measurements will be utilized in future experiments to establish the presence of mineral types, and to follow the change in lattice spacings of these minerals during cation exchange reactions, especially with cesium. Data procured from these measurements can be used to indicate the relationship between Cs availability and the extent of collapse of the mineral lattice.

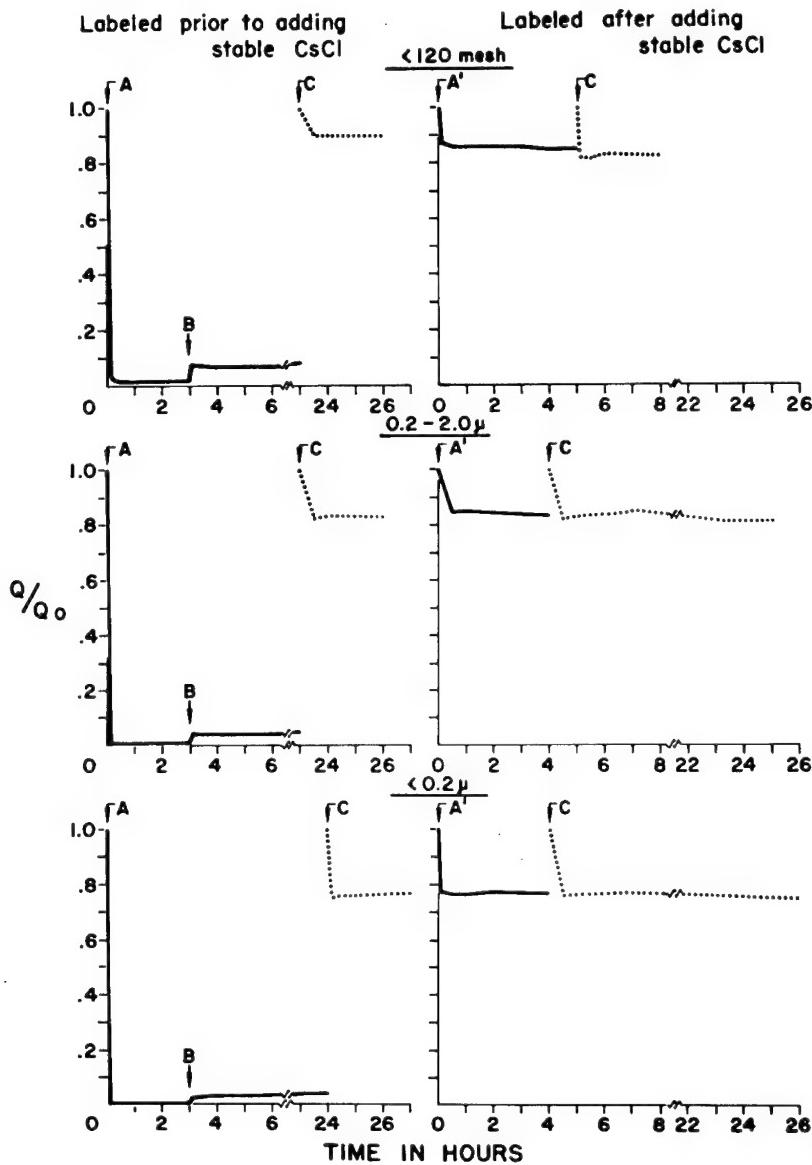


Figure 1. Sorption and desorption rates of  $^{134}\text{Cs}$  by  $< 120$  mesh,  $0.2\text{-}2.0 \mu$ , and  $< 0.2 \mu$  size fractions of Montana vermiculite where the initial labels were prior to and after the addition of one symmetry stable CsCl.

— Shows fraction of first aliquot  $^{134}\text{Cs}$  remaining in solution.

- - - - Shows fraction of second aliquot  $^{134}\text{Cs}$  remaining in solution.

A. Added first aliquot of  $^{134}\text{Cs}$  with no additional electrolyte.

A<sup>1</sup>. Added first aliquot of  $^{134}\text{Cs}$  after equilibrating with one symmetry stable CsCl overnight.

B. Added one symmetry of stable CsCl.

C. Added second aliquot of  $^{134}\text{Cs}$ .

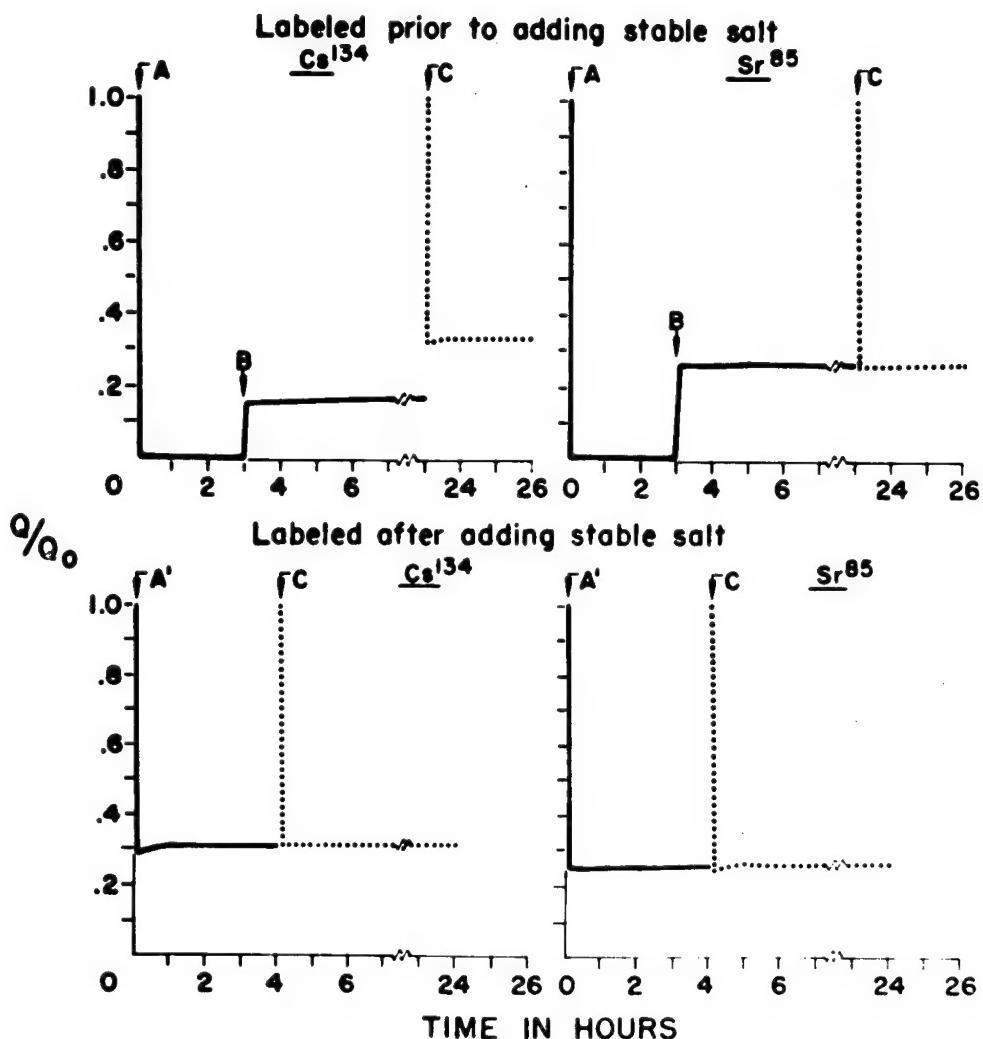


Figure 2. Sorption and desorption rates of  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  by 2-50  $\mu$  size fraction of Georgia kaolinite where the initial labels were prior to and after the addition of one symmetry stable CsCl and SrCl<sub>2</sub>, respectively.

— Shows fraction of first aliquot of isotope remaining in solution.

- - - - Shows fraction of second aliquot of isotope remaining in solution.

A. Added first aliquot of isotope with no additional electrolyte.

A<sup>1</sup>. Added first aliquot of isotope after equilibrating with one symmetry of stable salt overnight.

B. Added one symmetry of stable salt.

C. Added second aliquot of isotope.

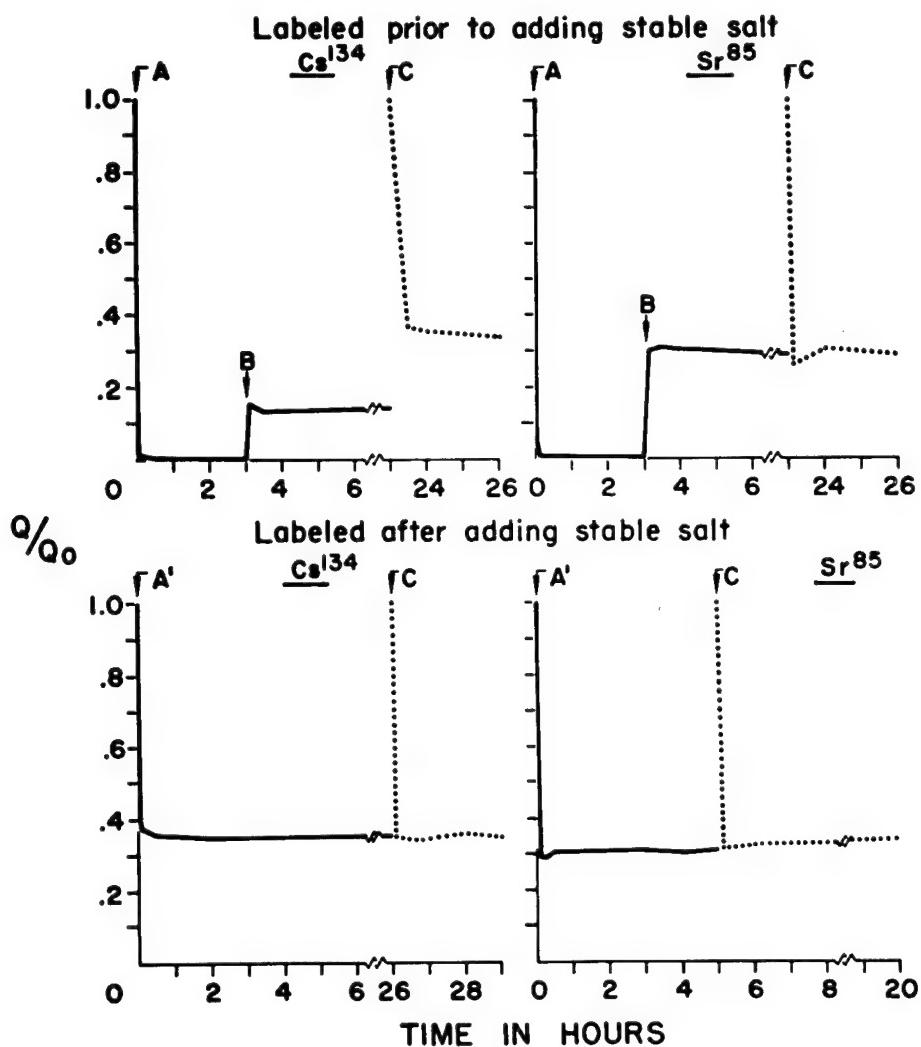


Figure 3. Sorption and desorption rates of  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  by  $< 0.2 \mu$  Wyoming bentonite where the initial labels were prior to and after the addition of one symmetry stable  $\text{CsCl}_2$  and  $\text{SrCl}_2$ , respectively.

— Shows fraction of first aliquot of isotope remaining in solution.

- - - - Shows fraction of second aliquot of isotope remaining in solution.

A. Added first aliquot of isotope with no additional electrolyte.

A<sup>1</sup>. Added first aliquot of isotope after equilibrating with one symmetry of stable salt overnight.

B. Added one symmetry of stable salt.

C. Added second aliquot of isotope.

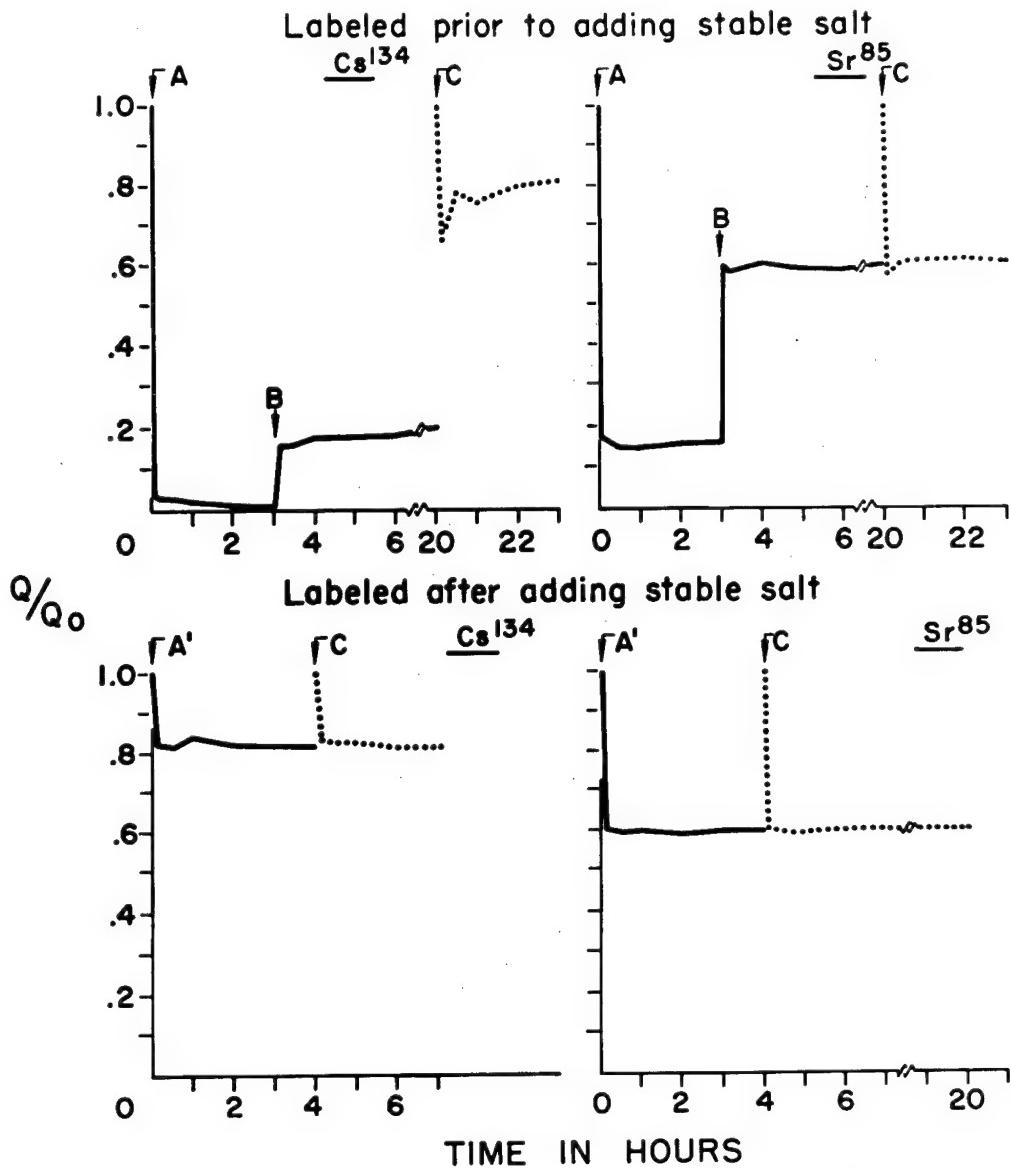


Figure 4. Sorption and desorption rates of  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  by  $< 150$  mesh Fithian illite where the initial labels were prior to and after the addition of one symmetry stable  $\text{CsCl}_2$  and  $\text{SrCl}_2$ , respectively.

— Shows fraction of first aliquot of isotope remaining in solution.

- - - - Shows fraction of second aliquot of isotope remaining in solution.

A. Added first aliquot of isotope with no additional electrolyte.

A'. Added first aliquot of isotope after equilibrating with one symmetry of stable salt overnight.

B. Added one symmetry of stable salt.

C. Added second aliquot of isotope.

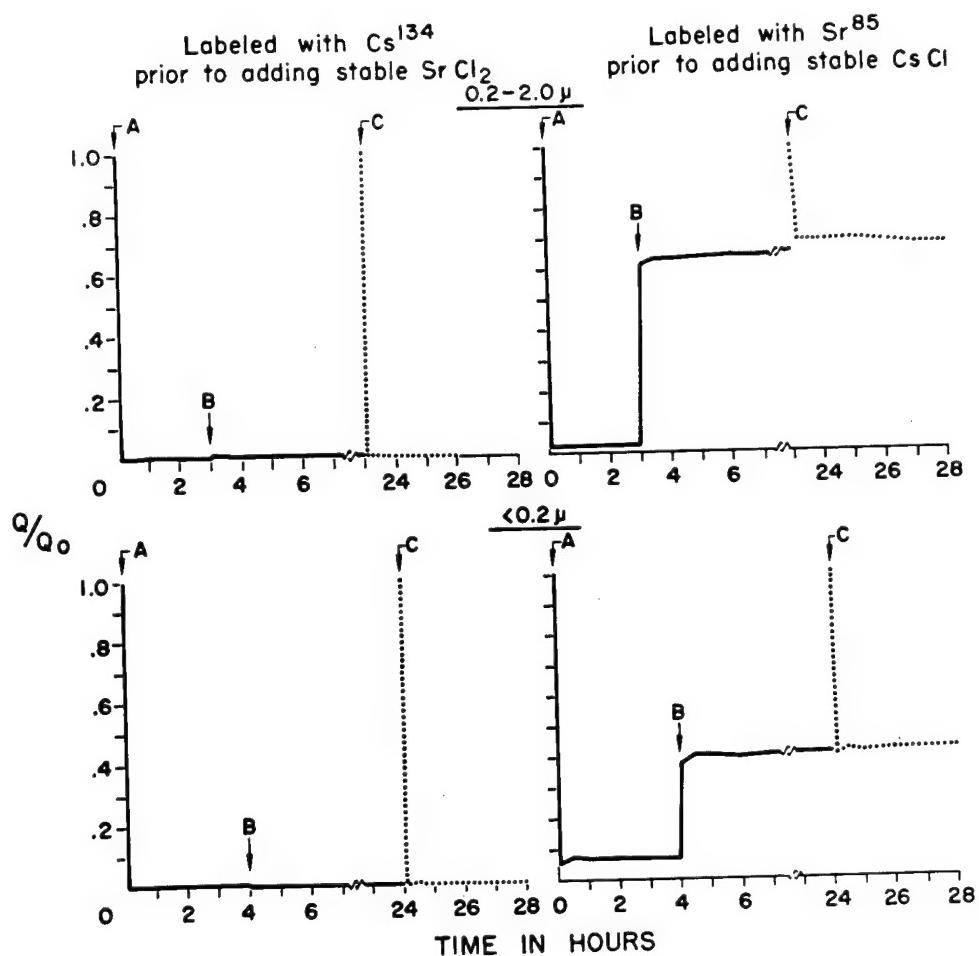


Figure 5. Sorption and desorption rates of  $^{134}\text{Cs}$  and  $^{85}\text{Sr}$  by 0.2-2.0  $\mu$  and < 0.2  $\mu$  size fractions of vermiculite where the initial labels were prior to the addition of one symmetry  $\text{SrCl}_2$  and  $\text{CsCl}$ , respectively.

— Shows fraction of first aliquot of isotope remaining in solution.

- - - - Shows fraction of second aliquot of isotope remaining in solution.

A. Added first aliquot of isotope with no additional electrolyte.

B. Added one symmetry of stable salt.

C. Added second aliquot of isotope.

## PLANT STUDIES

### Genetic Control and Environmental Modification of Seed Radiosensitivity

T. S. Osborne and M. J. Constantin

#### Scope:

One of the chief deterrents to more widespread use of radiation for inducing mutations in plant breeding programs has been the difficulty of establishing base lines for treatment. Even when the same kind of tissue is used from different species, this being generally dormant seeds, genetic differences between species can cause as much as a hundred-fold variation in dose response, and environmental factors operating during and after irradiation can cause a twenty-fold spread in response. As long as the factors causing such ranges of behavior were unknown hence uncontrollable, the frustrating situation existed where plant geneticists had to spend considerable time establishing dose response in their material and sometimes these efforts were undone, upon attempted repetition, by uncontrolled environmental fluctuations. We thus set about several years ago to study (a) the variations within dormant seeds among many species in their histological organization and biochemical factors, as correlated with their sensitivity or resistance to radiation, and (b) factors of environment, such as seed moisture and the gaseous atmosphere around seeds during and after treatment, which could alter the basic genetic response to radiation.

An understanding of such factors would greatly augment the desirability of inducing mutations in routine breeding programs. It would further contribute to basic understanding of radiobiological phenomena, hence be of potential application to such disparate fields as cancer therapy and space travel.

In a typical experiment seeds are equilibrated in forced-air systems previously described<sup>1/</sup> at one of five relative humidities. Following equilibration, which generally requires two or three weeks, seeds are subdivided and exposed to one of five radiation treatments (plus one or more untreated control) and are then further subdivided into four replications, generally of 100 seeds each. Exposures are made with seeds packed in dry ice, when oxygen and storage effects are desired to be constant or controlled. After all seeds have been treated, the packages are immediately immersed in boiled water, then seeds are distributed in sterile peat-perlite medium in randomized complete blocks and grown in

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<sup>1/</sup> Plant Physiol. 36:309, 1961.

controlled-environment growth rooms previously described. Each datum is the dry weight of true growth--above ground for monocots and above the cotyledonary node for dicots--after an interval arbitrarily chosen for each kind of plant. This interval may range from 6 to 25 days for different species.

After harvest the data are submitted to analysis of variance, then dose response is programmed for expression as probits relative to untreated controls measured against logarithm of dose.

When sufficient species have been studied, partial and multiple correlations may be performed where the single dependent variable ("Y") is the so-called  $D_{50}^{1/2}$  and the independent variables ("X's") are the embryonic factors believed to contribute to radiosensitivity. Obviously each kind of radiation, such as gamma rays or neutrons, must be studied separately. Variation can then be partitioned and the proportions attributable to each X can be estimated.

Status:

As of the last report we had measured and counted numerous histological variables in dormant embryos of several species and correlated these findings with the maximum radiation tolerance--wherein environmental conditions known to be important were also experimentally varied--of the species to gamma rays. The most important variable in our experience is the average volume of nuclei in the apical meristem of dormant embryos, with other factors contributing lesser amounts to innate radiation response.

Effects of fission neutrons, and their genetic control in seed systems, have been under study for a much shorter interval, and at the last report a tentative partitioning of variance and a lesser knowledge of environmental factors were at hand. We had earlier attempted to estimate absorbed dose of neutrons by seeds from measured delivered exposure by means of correction factors calculated from some crude published figures for different kinds of seeds; the resulting factors ranged from 0.54 to 0.72. Precise microchemical determinations permitted us to calculate more accurate correction factors which turned out to vary both below and above unity, ranging from 0.83 to 1.23 and averaging 1.02.

Progress during this report period:

When the proportions of variance are partitioned as described above, the factors influencing response of dormant embryos to gamma

1/ Maximum exposure tolerated by resting seeds to reduce subsequent seedling growth, measured as dry weight, to 50% of untreated controls.

rays and neutrons may be compared as in Table 1. It will be noticed at once that although the same 10 species are compared in each case, the total variance accounted for in the case of gamma rays is more than 97% but for neutrons is less than 87%. It is further obvious that the average nuclear volume seems considerably less important to fission neutrons than to gamma rays, whereas the reverse is true for the slope of moisture vapor uptake prior to irradiation. One further surprise is the remarkably high importance of terminal apex volume to neutrons, accounting for more than 15% of the total variance, whereas the same feature accounted for less than 1% of the variance in the case of gamma rays. Addition of more species and further refinements of technique will undoubtedly alter these figures, which should be considered in a relative way only.

When  $D_{50}$  values are obtained on the same kinds of seeds for gamma rays and for neutrons, the former can be divided by the latter to establish a datum called "relative biological efficiency" or RBE. While there is some dispute over the significance of this item, and we will not discuss its possible ramifications here, the fact remains that RBE values for total-body exposure of small and large animals are generally less than 10 and cluster around unity. By contrast our current results can be seen in Table 2 in which the RBE's range from 12 to 56 averaging about 36. It should be remembered that the values for gamma rays represent the highest possible resistance with varying environmental conditions, thus the numbers stated represent maximum RBE's.

When microchemical analysis of seeds was performed permitting calculation of correction factors to convert administered dose to absorbed dose (see ORO 648), it was possible for the first time in our experiments to obtain accurate measurements of absorbed dose of fission neutrons. A simplification became possible when it was found that these correction factors are almost perfectly correlated with content of hydrogen alone in the seeds (see Figure 1). Two points should be made: the chemical analyses were performed on entire seeds rather than only on embryos, thus are open to some criticism, although it may be argued that proton ejection from within and from without the embryo will effectively counterbalance each other. One could, however, judging from Figure 1, obtain quite accurate correction factors for estimating absorbed dose by analyzing only for hydrogen in seeds or embryos.

Further experiments and refinements permit us to compare  $D_{50}$  values for each species in response to gamma rays and to unmoderated fission neutrons, as shown in Figure 2. The correlation while extremely high is certainly not perfect, and the reasons for differing sensitivity to neutrons and to gamma rays constitute an important feature necessitating further research.

Discussion:

With a few more neutron experiments it should be possible to describe intelligently the various genetic and environmental influences affecting response of dormant seeds to radiations. The remaining task in this regard will be to perceive the chromosomal and point mutational effects of these mutagens, some experiments concerning which are already well underway. Distinctions between quantitative and qualitative mutations must be and are being sought. An additional important dimension will be added when chemical mutagens are brought in for comparison with gamma rays and neutrons from the three viewpoints of damage to seedling growth, chromosomal aberrations, and measurable mutations. Such information should be of inestimable value in aiding plant geneticists to determine the preferred kind, amount, and method of mutational treatment, as well as eliciting significant fundamental radiobiological information.

TABLE 1. Proportions of Variance Attributable to Various Embryonic Components; Seeds Irradiated with  $^{137}\text{Cs}$  Gamma Rays or Fission Neutrons; Total of 10 species

Embryonic Trait	Per cent of Variance	
	Gamma Rays	Neutrons
Log <sub>10</sub> average nuclear volume	78.73	59.03
Slope of water vapor uptake	10.37	3.21
Nuclear volume per chromosome	3.55	2.27
Number of apical initial cells	2.23	0.77
Number of primordial leaves	1.35	1.65
Volume of terminal apex	0.70	15.56
Number of chromosomes	0.19	3.06
Number of terminal apical cells	0.18	0.89
Total accounted for:	97.30	86.44

TABLE 2. Relative Biological Efficiency (RBE) of Fission Neutrons to  $^{137}\text{Cs}$  Gamma Rays in terms of Absorbed Dose by Dormant Seeds Necessary to Reduce Subsequent Seedling Growth by 50 per cent ( $D_{50}$ )

Code No.	Genus and species	$D_{50}$ Gamma (kilorads)	$D_{50}$ Neutrons (kilorads)	RBE (r/N)
1	Allium cepa	16.9	0.490	34.5
2	Brassica napus	148.0	3.135	47.2
3	Cucumis sativus	64.0	1.364	46.9
4	Festuca arundinacea	15.8	1.100	14.4
5	Gossypium arboreum	14.2	0.469	30.3
6	Hordeum vulgare	27.4	0.587	46.7
7	Lactuca sativa	30.0	1.000	30.0
8	Linum usitatissimum	75.9	6.272	12.1
9	Lycopersicon esculentum	60.0	1.481	40.5
10	Trifolium incarnatum	165.7	2.980	55.6
			(Av.)	(35.8)

**Correction Factor, Delivered to  
Absorbed Dose, Neutrons**

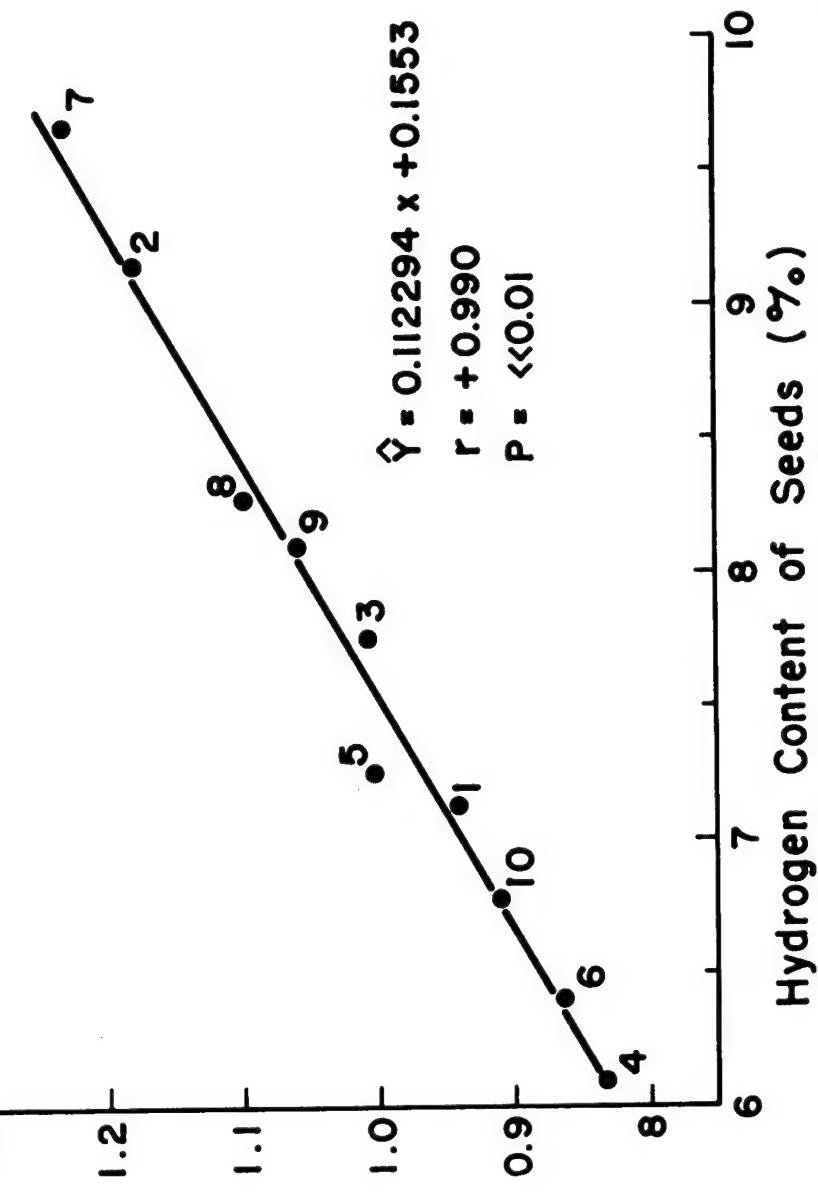


FIGURE 1. Almost perfect correlation of seed hydrogen content (for seeds from 10 genera) with correction factor necessary to calculate from "rads, tissue dose in air" to "rads absorbed by seeds".

**D<sub>50</sub> NEUTRONS  
(kilorads)**

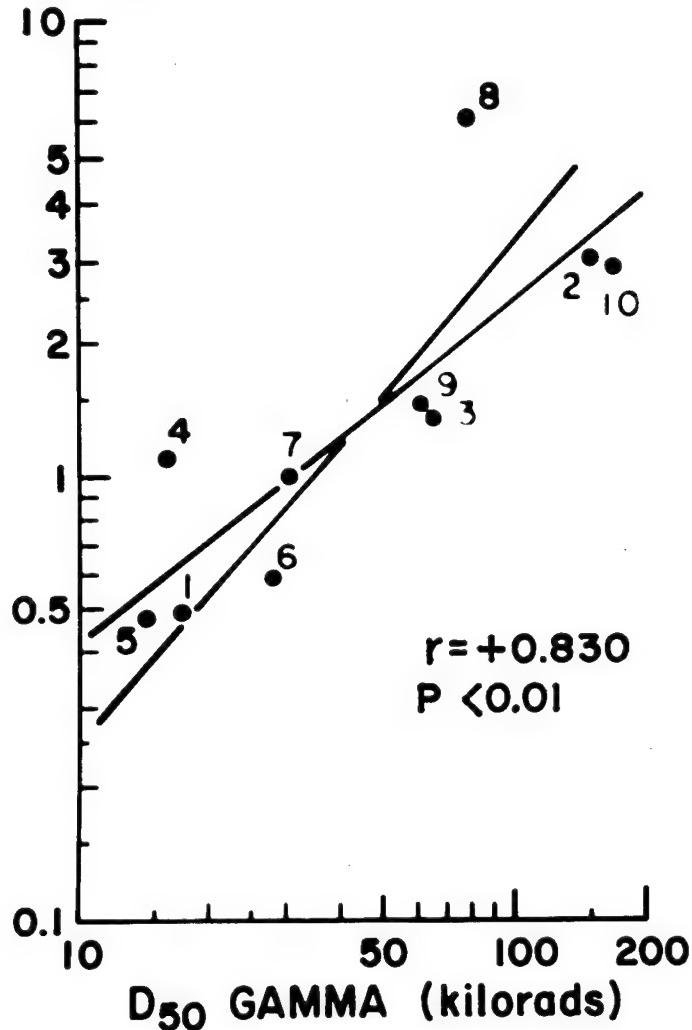


FIGURE 2. Relationship between D<sub>50</sub> for gamma rays, for seeds at their most radioresistant state, and D<sub>50</sub> for fission neutrons. (1, *Allium cepa*; 2, *Brassica napus*; 3, *Cucumis sativus*; 4, *Festuca arundinacea*; 5, *Gossypium arboreum*; 6, *Hordeum vulgare*; 7, *Lactuca sativa*; 8, *Linum usitatissimum*; 9, *Lycopersicon esculentum*; 10, *Trifolium incarnatum*.)

## Mutations in Quantitatively Inherited Traits

M. J. Constantin and T. S. Osborne

### Scope:

Many of the traits that are of utmost interest to breeders are quantitatively inherited, showing continuous variation and being governed by many genes. Such traits are sometimes called metrical or polygenic, and include many features of importance to plant and animal breeders including size, yield, and early maturity. In contrast, qualitatively inherited traits show discontinuous variation and are governed primarily by single major genes, examples being coat color in some animals and resistance to certain diseases in plants. Needless to say, there are gradations in some cases between the two types of traits.

Irradiation causes mutations some of which can be utilized to improve economic organisms. The most frequently observed and striking mutations are those affecting qualitative traits which can be identified in a single plant. However, such mutations generally have deleterious side effects including decreased viability and vitality.

From present data, it appears logical that mutations affecting one or a few genes governing quantitative characters probably occur just as frequently as those affecting major genes. However, more elaborate and sophisticated detection techniques are necessary to identify quantitative changes. Large replicated populations must be subjected to statistical analysis to determine the direction and magnitude of the change. Mutations involving quantitative characters are less drastic physiologically and morphologically than qualitative gene mutations. Thus, they generally do not decrease viability and vitality. Comparatively, there has been a greater number of studies devoted to mutations affecting qualitative traits. Yet there appears to be a greater potential benefit to be derived from studies of mutations affecting quantitative characters such as yield, date of maturity, and the many facets of quality.

### Status and Progress During Report Period:

Studies of radiation effects on quantitative characters in American Upland cotton (Gossypium hirsutum L.) and soybeans (Glycine max L.) have been conducted. The effects of different levels of gamma radiation from cobalt-60 and different genotypes within each species on the frequency and nature of mutations have been emphasized. Character evaluation was done under a system of codes to minimize bias.

Cotton. Resting seeds of two southeastern and two southwestern advanced strains of cotton were exposed to 10, 20, or 30 kr of gamma radiation and were grown in the field. A control population was grown and handled similarly except that the seeds were not irradiated. Cotton is an often-cross-fertilized plant; therefore, enforced selfing was used in the advancement of generations.

Approximately 1400 M<sub>1</sub> plants were selfed from which slightly over 700 boll-to-row M<sub>2</sub> progenies were grown. It is presumed that radiation-induced self-sterility combined with the adverse effects of bagging caused many of the selfed ovaries to abort. A random sample of M<sub>2</sub> plants were grown as M<sub>3</sub> plant-to-row progenies. Selection based on data for fiber length, strength, fineness, and elongation was imposed on M<sub>3</sub> and later generations. The material was also scored for lint percent and weight/100 seeds.

Fiber data from M<sub>2</sub> and M<sub>3</sub> populations indicated that changes in these quantitative traits were induced by seed irradiation. Statistical variance showed changes in positive and negative directions compared to the control. There were significant treatment effects in 7 of 12 combinations involving fiber length, strength, and fineness in the M<sub>2</sub> generation. Only one of these combinations showed a significant change in the M<sub>3</sub> generation. This could be due to higher variance induced by environment as was observed in the controls and by continued inbreeding.

A high-low selection approach was used through the F<sub>5</sub> generation in irradiated and control populations. With continued selfing, there was a tendency for irradiated lines to approach the expression of the controls. It was decided that select high and low performing lines from irradiated populations would be crossed with their counterparts in the control population. Crossing was done within the respective strains, but only the treatment producing the greatest differential of expression for any one trait was used. Reciprocal crosses were made in case of possible maternal inheritance and the F<sub>1</sub> populations were grown during the winter of 1965-66 at Iguala, Mexico. This year (1966) the parental lines, reciprocal F<sub>1</sub> plants, and their respective F<sub>2</sub> progenies are being grown at the West Tennessee Experiment Station. A perfect stand of plants was obtained in all populations and, barring adverse weather during harvest, we should have a very good crop. A visual observation of the growing plants showed no indications of extreme variation in plant type. The data from this planting will be used in an attempt to determine whether the phenotypic changes observed in the quantitative traits were due to genotypic alteration, and, if so, how many genes were altered to effect the change observed.

Soybeans. A similar plan of selection for the quantitative traits, high bean protein and high bean oil has been carried on for several years, with chemical determinations generously provided by the Regional Soybean Laboratory at Urbana, Illinois. In 1965, while the  $M_9$  plants were growing, a routine check of the previous 5 years' data showed the population means tending to converge (see ORO-635). For 1966 plantings,  $M_{10}$  seeds were planted alongside the earliest viable seeds of their progenitors: either  $M_4$  (1960) or  $M_5$  (1961) seeds. Parallel selections will be made for 3 or 4 years in the ancestor and descendant lines in an effort to see whether the convergence is caused by ineffectual selection, changes in gene frequency, or human error.

Analysis of variance showed significant selection-by-year interactions; Figures 1 and 2 depict the yearly performance of a few selections relative to the means of all selections for the years of concern. It will be noted that any departure from parallelizing the mean for all varieties (heavy dashed line) indicates an interaction.

Since this study involves only a modest use of time and labor, it will be continued for perhaps 3 years in attempting to answer the original questions, namely, (1) can a single seed irradiation significantly alter quantitatively inherited traits (oil, protein) in soybean? (2) When the highest and lowest lines for each trait within a variety are effectively separated, can the number of genes altered be estimated? And an unforeseen new question: (3) when means of high and low lines tend to converge over a 5-year period, what is the cause?

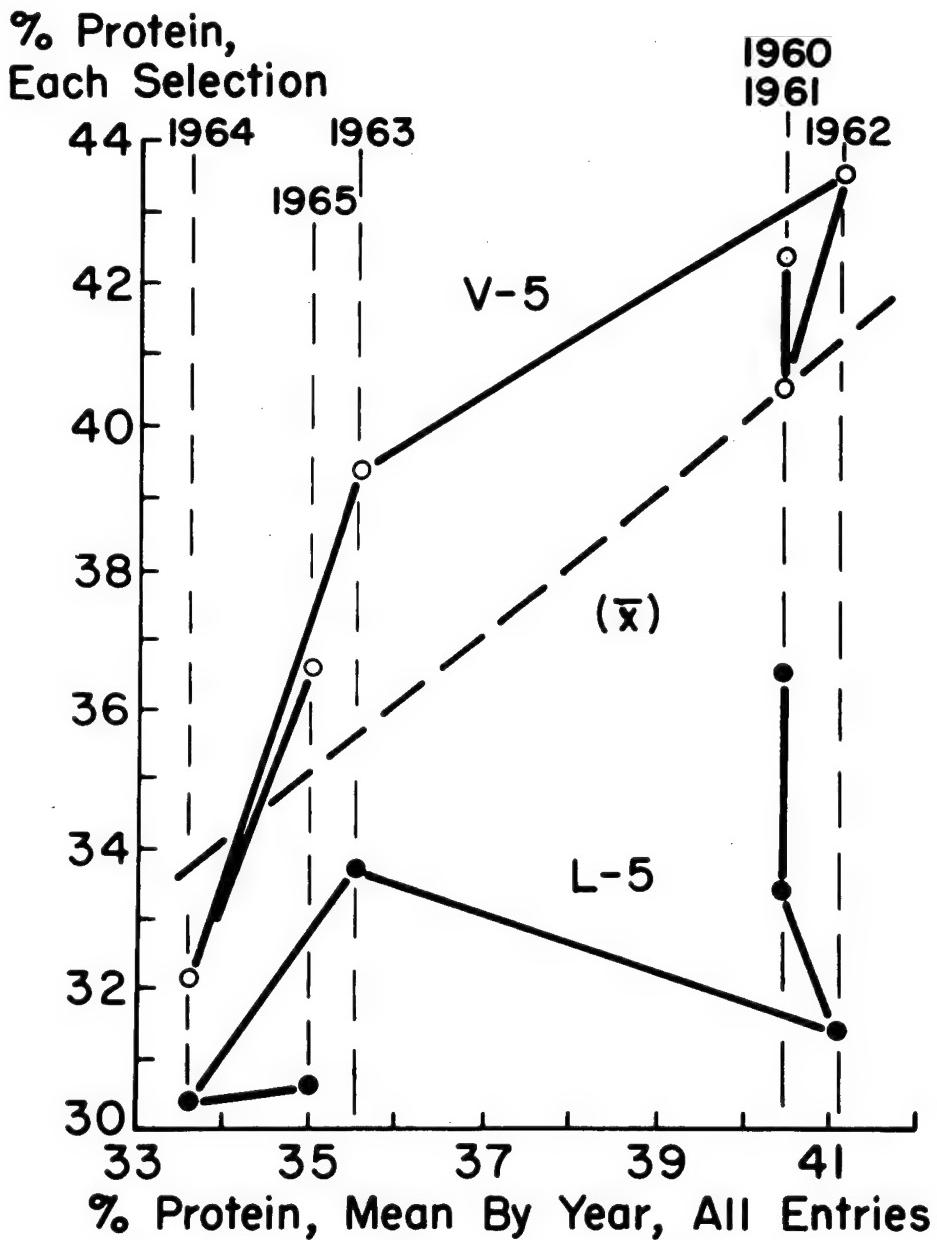


Figure 1. Protein content of 2 soybean selections showing yearly variation and interaction; Y, value of individual selection; X, average value of all selections by year.

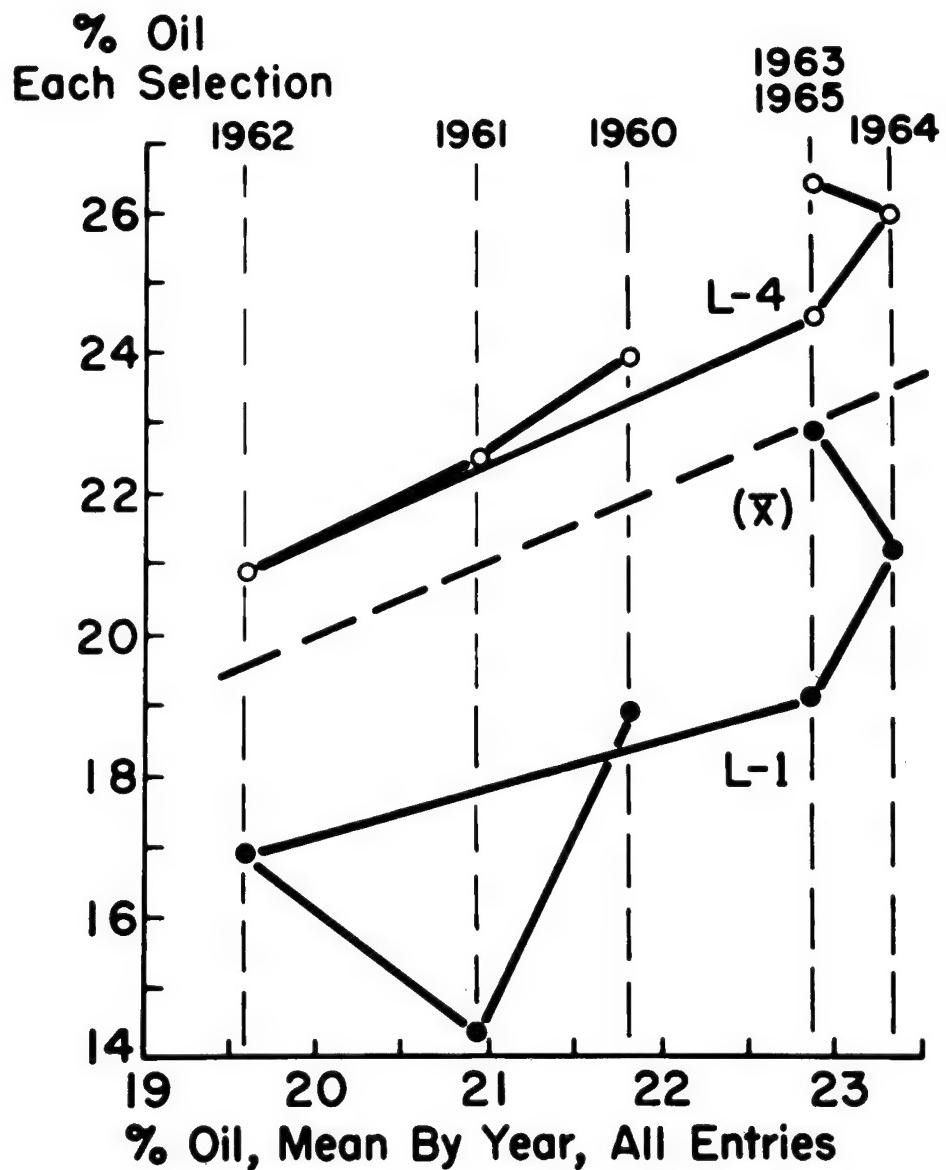


Figure 2. Oil content of 2 soybean selections showing yearly variation and interaction; Y, value of individual selection; X, average value of all selections by year.

## Irradiation of Interspecific Hybrid Zygotes and Proembryos

M. J. Constantin and T. S. Osborne

### Scope:

It was proposed that radiation-induced chromosomal translocations might permit the exchange of genes from one parental genotype to another in the case of interspecies hybridization where natural genetic barriers exist. Interspecific hybrids of Gossypium hirsutum L. and G. barbadense L. were selected as test organisms. It was realized from the onset that many of the problems inherent to interspecific crosses (e.g., poor germination, seedling inviability, and sterility) are also induced by irradiation.

### Status:

American Upland (Gossypium hirsutum L.) and Sea Island (G. barbadense L.) are two closely related species of cotton that are grown commercially. Cotton breeders and geneticists have been unsuccessful in their attempts to develop a stable hybrid variety possessing the agronomic desirability of American Upland and the high-quality fiber of Sea Island. Both species are allotetraploids ( $2N = 52$ ), and the  $F_1$  hybrid shows good chromosome homology, is fully fertile, and possesses attributes of each parental species. However, a genetic breakdown occurs in the  $F_2$  and succeeding generations as manifested by varying degrees of sterility, extreme late maturity, low viability, and a preponderance of quasi-parental types with inferior fiber quality. The phenomenon has been observed repeatedly and has been attributed to one or more causes including cryptic structural differences between parental chromosomes and induced imbalance between major and minor (modifier) genes or gene complexes.

We proposed the use of irradiation to cause chromosomal breaks followed by translocations as a means of possibly circumventing the genetic breakdown. The idea was that translocations would permit the incorporation of some Sea Island chromatin into the American Upland genetic background. Presumably the traits governed by genes involved would be stable in the translocation homozygote.

We chose to irradiate the interspecific hybrid zygote because all chromosomes of each parent are together within a single nucleus and available for translocations, and the problem of sectorial chimeras that result from the irradiation of multicellular tissue is avoided. Because of variation in the time of fertilization and zygotic division in ovules within the cotton boll, early proembryos as well as zygotes are probably present at time of irradiation. We postulated that the probabilities of recovering desirable transloca-

tions would be considerably higher if a large  $F_1$  population (approximately 10,000 irradiated individuals) were grown.

The identification of translocations by cytogenetic analyses of that many  $F_1$  plants did not appear feasible. Instead it was decided that a comparison of statistical variance for fiber length, strength, fineness, and elongation within  $F_2$  populations of irradiated and non-irradiated  $F_1$  plants would be used to identify populations with stable genetic transfers.

There are several plant breeding methods that could be employed with a problem of this nature. Continued enforced selfing (Pedigree) without selection was used to provide basic data pertinent to the frequency of translocations and their influence on the genetic breakdown and the expression of traits. Open pollination with mass selection was also used in an attempt to let nature take its course and then impose selection pressure in advanced populations after the more undesirable phenotypes have presumably been eliminated. Any select line isolated in this manner will be examined cytologically for evidence of translocations.

Preliminary results indicated that the interspecific hybrid remains in the zygotic stage through day 4 or 5 after pollination and the zygote will tolerate up to approximately 1 kr of gamma radiation. Instead of 10,000 irradiated hybrid plants to be grown during a single growing period, approximately 1,000 have been grown over a three-year period.

In the  $F_2$  and  $F_3$  generations under enforced selfing, we have observed a most discouragingly high frequency of low seed germination and seedling survival. In addition most of the survivors showed partial to complete self sterility. For example, approximately 40,000  $F_2$  and  $F_3$  flowers have been selfed and approximately 1% have produced seeds.

Statistical analysis of fiber data have shown that there are differences among irradiated  $F_1$  plants which in theory should be highly uniform in the absence of irradiation. The control plants showed the expected degree of uniformity. Fiber data from  $F_2$  and  $F_3$  show a decrease in population means in both irradiates and non-irradiates which is as expected due to continued selfing (inbreeding). Preliminary statistical analyses of the few  $F_2$  and  $F_3$  populations from individual  $F_1$  plants (referred to as families) with large enough numbers for significance indicate that variance does not differ sufficiently to indicate translocations (stabilization). It is felt at present that pedigree breeding program has yielded  $F_2$  and  $F_3$  populations from which we are unable to conclude whether translocations have occurred.

The material that has been advanced under open pollination and mass selection has performed better than the selfed material. As in the selfed populations,  $F_2$  and  $F_3$  plants showed rank vegetative growth with very low yield, but there was apparently sufficient cross-fertilization for perpetuation. The material had stabilized sufficiently by the  $F_4$  generation that selection based on agronomic type was imposed. Approximately 25 percent of the population met the basic selection criteria and these are being grown as  $F_5$  lines in 1966. Fiber analysis of the selected lines showed that some lines have fibers that are long, strong, and high in elongation--a combination of traits not readily available in commercial cotton. It is not known how stable these traits are and this will be of considerable interest in the future.

Progress During Report Period:

During this report period little was done other than to plant the open pollinated selections and the last  $F_3$  population from enforced selfing. Observation of the  $F_5$  open pollinated selected lines showed an amazing degree of uniformity within most lines and the degree of fruiting in some lines is most encouraging. On the other hand, the last  $F_3$  population from enforced selfing resembles the previous two populations and little hope is held that its performance will differ significantly. The reason(s) for the observed differences between selfed and open pollinated material are not fully understood.

Discussion:

It is suggested now that further testing of the selfed material be discontinued and the fiber be stored for possible future analysis. In the meantime, any  $F_4$  selfed seeds should be grown next year and selection should be imposed rigidly for agronomic type. Only these few selected lines should merit continued attention. The open pollinated material should be advanced as originally planned.

## Radiation Effects on Horticultural Plants

J. E. Love

### Scope:

One phase of the plant research is concerned with radiation effects on horticultural plants. These plants are especially adapted for radiation studies because of their heterozygous nature and/or chimeric nature. There is considerable interest at the present time in the use of radiation to produce sports or bud mutations, and research in this area is increasing. Radiation-induced mutants, like other bud mutations, may be perpetuated by asexual reproduction.

Studies have been established to evaluate the morphological and physiological changes that lead to abnormal growth or permanent growth modifications. Information concerning the radiation effects of several species is used to further the knowledge on mutation induction in vegetatively propagated plants.

### Status:

To date, approximately 25 species of woody and herbaceous ornamental plants have been exposed to gamma rays and fast neutrons. A comparison based on survival and growth reduction among the species indicated that fast neutrons are approximately 15 times more damaging to rooted cuttings than are gamma rays. Whereas the tolerances for fast neutrons ranged between 50 and 1,500 rads, the tolerances for gamma rays fell between 2,000 and 20,000 rads.

Radiation-induced mutations have been produced in several species. These mutations have been represented by changes in leaf and flower pigments and changes in plant size. Many of the modifications in plant type have been attributed to specific changes in the growing point of a bud (both genetic and nongenetic).

The mutation frequency for certain ornamental species (Coleus and Euphorbia) is extremely high after exposure to fast neutrons. For example, approximately 90 percent of the Coleus plants exposed to 1,000 rads of fast neutrons exhibited a bud mutation. In addition, Coleus and Euphorbia have produced more than one type of bud mutation, sometimes on the same plant. Both species will be used extensively as the experimental plant material in future experiments concerning mechanisms of radiation action.

Progress During Reporting Period:

Metabolic changes associated with mutation induction in Coleus

Blumei: Most of the bud mutations produced in Coleus cv. 'Scarlet Red' have involved an alteration in the synthesis of anthocyanin pigments (Proceedings American Society for Horticultural Science 88:627-630, 1966). One bud mutant (MUTANT 1) appeared much darker than the control plants and another was almost completely void of the red pigment (MUTANT 2). Thus control plants and mutant plants differed considerably in the amount of pigment produced and have provided an excellent system to study metabolic changes associated with somatic mutation.

The leaf tissues of the control and Mutant-1 plants contained 2 anthocyanin pigments (Table 1) tentatively identified as delphinidin 3, 5-diglucoside and cyanidin 3, 5-diglucoside. Separation and identification of the pigments in each plant type were made with the aid of paper chromatography and adsorption curves of the pigment extracts. Cyanidin diglucoside was the major fraction and was about 9 times the concentration of the minor fraction. Total pigment in Mutant-1 was approximately twice that of the Control plants; however, the two fractions were in the same relative proportion in each plant type. Anthocyanin pigments were not observed on chromatograms prepared from leaf tissues of Mutant-2.

In Figures 1a-1g, several comparisons are made between control and mutant plants. Samples of leaf tissue were analyzed for various biochemical constituents including reducing sugars, protein, anthocyanin pigments, chlorophyll, percent dry weight, nitrogen and pH. Means were compared using individual degrees of freedom comparison with an "F" test to denote significance.

Alteration of anthocyanin synthesis due to somatic mutation was usually followed by a significant change in other plant metabolites. This was especially true in comparisons between Control and Mutant-2 plants. These data would indicate that the plant cell acts as a metabolic pool and changes in one cellular constituent is usually reflected by concurrent changes in other constituents.

In order to test this theory further, we have investigated the participation of the pentose phosphate pathway (PP) in anthocyanin development. By increasing the PP pathway, shikimic acid, a major precursor in anthocyanin synthesis, should be increased and more red pigment produced. (Shikimic acid is a condensation product of erythrose and phosphoenol pyruvate, while erythrose is a product of the PP pathway.) Activity of the PP pathway was estimated by determining the so-called C<sub>6</sub>/C<sub>1</sub> ratio by using specifically labeled glucose (G-6-C<sup>14</sup> and G-1-C<sup>14</sup>).

Decarboxylation of the G-1-C<sup>14</sup> glucose was higher and G-6-C<sup>14</sup> lower in leaf tissues of the control plants than in Mutant-2. The C<sub>6</sub>/C<sub>1</sub> ratio was therefore lower in the Control (.483) than in Mutant-2 (.644) indicating a strong PP activity. The C<sub>6</sub>/C<sub>1</sub> ratio was even lower for Mutant-1 (.470). Thus, mutation induction has not only altered the content of certain cellular constituents, but has markedly changed the pathway by which these components are utilized.

Mutation Induction in Euphorbia: In a previous progress report, we discussed the induction of chimeric bracts in three cultivars of poinsettias (ORO-648). After scoring for bract color, flowers were removed and plants from the 300 and 400 rad treatments were subjected to additional artificial light to induce vegetative growth. Approximately 3 to 4 "breaks" (growth of axillary buds) were produced on each plant. Finally the plants were flowered for a second time using black-cloth shading. New growth from the axillary buds was scored for mutations involving the size, shape, and color of leaves and bracts.

At least 4 types of bud mutations have been produced with fast neutron irradiation. These have been categorized according to the character induced (Table 2).

Sectorial Bracts: Sectorial bracts produced during the second flowering cycle were the same color, but were usually larger than those produced earlier.

Leaves with Chlorophyll Variegations: Several variegated leaf patterns occurred in new growth (under a long-day light regime). Numerous variegated patterns occurred in 'Ecke Pink' and 'Ecke White' while only one chlorophyll mutant was observed in 'Indianapolis Red'. Stewart (Genetics 52:925-947) showed that some chlorophyll variegations in Euphorbia are due to a change in genetic material carried in the plastids. Evidently the genetic material of the chloroplasts, like that of the nucleus, is susceptible to radiation-induced changes.

Mutations for Leaf and Bract Morphology: Irradiated plants produced numerous bud mutants involving the size, shape, and texture of the leaves. In one case, a modification in bract size was observed. An alteration in the ontogeny of leaf tissues through the selective destruction of 1 or more cell layers in the shoot meristem can be used to explain at least 2 mutants. This would appear to be the case in 'Ecke Pink' where some abnormal leaves had red petioles. The appearance of red petioles in the pink cultivar indicated an expression of the internal tissues. Another type of leaf mutant resulted from an absence of the palisade cells. This type of leaf mutant would probably indicate

a selective destruction of an inner cell layer since the second histogenic layer gives rise to the palisade cells.

Origin and Development of Modified Growth: Most of the color changes which resulted from irradiation of the terminal buds appeared sectorially within a single bract. The only exceptions were the pale- and dark-red sectors in 'Ecke Pink' where entire bracts and sometimes secondary flower branches were involved.

In contrast to the changes in terminal buds, radiation-induced changes in growth of the axillary buds involved sectors of/or entire branches. Individual branches were observed with several variegated patterns and with various leaf sizes and shapes. These modifications, however, developed under a long photoperiod where new leaves were continually being formed. Another factor governing the size of mutant sectors probably involved a difference in anatomy of the terminal and axillary buds at the time of irradiation. It is doubtful that the axillary buds were as advanced ontogenetically as the terminal buds, so induced changes in axillary buds resulted in larger sectors.

#### Discussion:

We will continue to work with the metabolic changes associated with the induction of somatic mutations. Experiments with the Coleus mutants will be concerned with the synthesis of anthocyanin pigments. An obvious question to be answered now is where the "block" or "shunt" occurs in the conversion of shikimic acid to anthocyanins or other polyphenols.

Irradiation experiments will also be continued with poinsettia. Results to date have been promising, and radiation may provide an important tool in the production of new cultivars. Several cultivars and new seedlings (of a known genotype and cell layer composition) will be irradiated this fall. The histology of at least one cultivar will be studied after irradiation.

TABLE 1.  $R_F$  Values of The Anthocyanin Pigments Extracted From Control and Mutant Coleus Plants

	$R_F$ (in BAW)	Color	Approximate percentage
Band 1	.14	Purple	10
Reported by Harborne (Delphinidin 3, 5-Diglucoside)	.15	Purple	
Band 2	.29	Magenta	90
Reported by Harborne (Cyanidin 3, 5-Diglucoside)	.28	Magenta	

BAW = n-butyl alcohol:acetic acid:water, 4:1:5 v/v.

TABLE 2. Radiation Induced Changes in Euphorbia Pulcherrima

Character Induced	Cultivar <sup>1/</sup>	Radiation Dose (rads)
Bract color (with cyathia)	1, 2	100-400
Leaf morphology	1, 2	300-400
Chlorophyll variegation	1, 2, 3	100-400
Early flowering	3	400

Cultivar 1 = Ecke Pink

2 = Ecke White

3 = Indianapolis Red

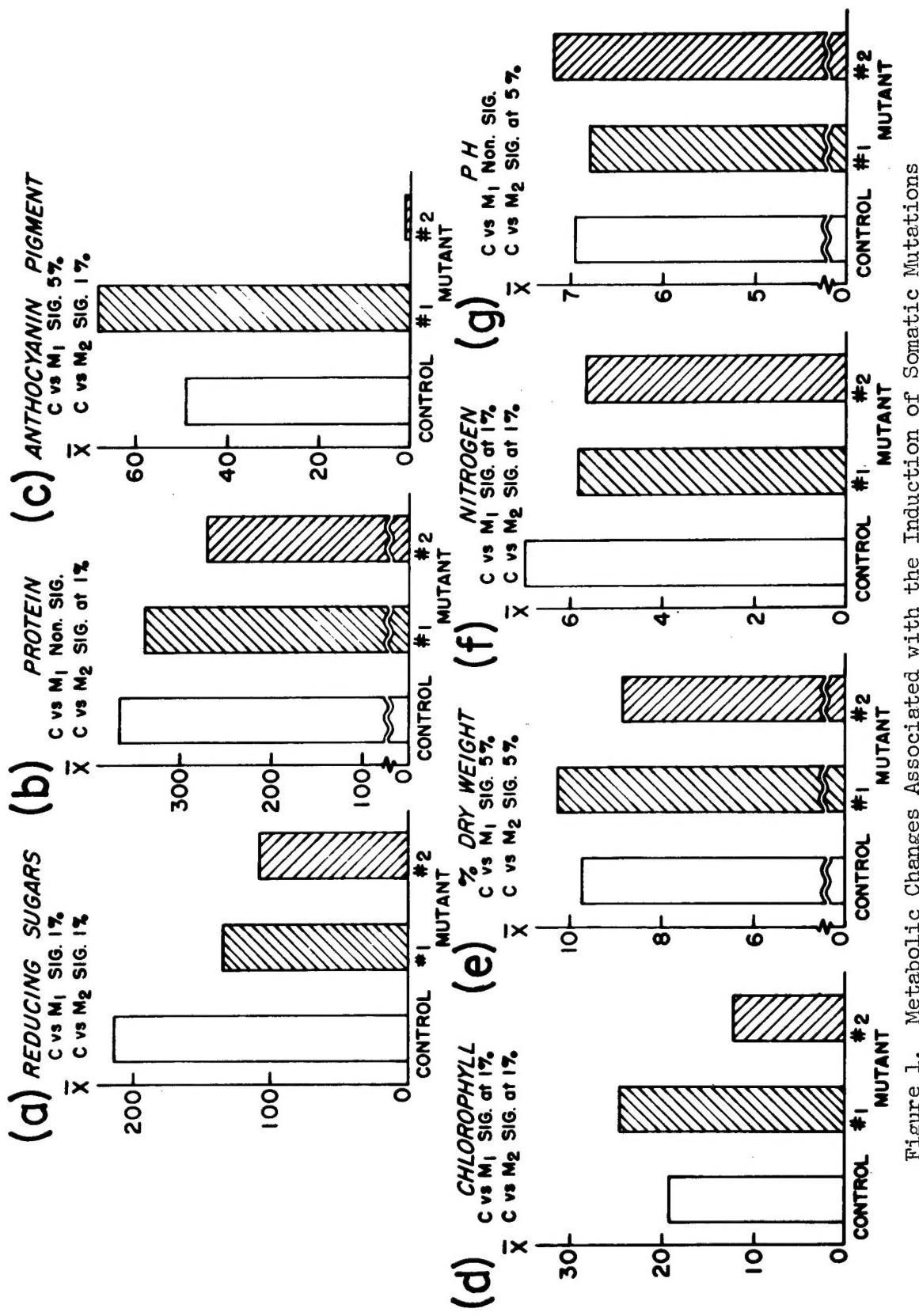


Figure 1. Metabolic Changes Associated with the Induction of Somatic Mutations in Coleus Blumei

PUBLICATIONS

Clinical Effects of Anesthesia Produced by Alternating Electrical Current

Charles E. Short  
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